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EDITORS

HENRY CHANDLER COWLES AND JOHN MERLE COULTER

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ERRATA

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- P. 5, center heading "Description" and following tabular material should follow line 6 on p. 6
- P. 11, line 9, for uredinal read uredinial
- P. 12, text fig. 2 legend, omit Photomicrograph of
- P. 19, line 10, for *pseudobalsameum* read *pseudobalsameum*
- P. 198, inscription on left of figure, for *oi*, read of



THE
BOTANICAL GAZETTE

March 1927

COMPARATIVE STUDY OF SPERMOGONIA OF
RUSTS OF *ABIES*

LILLIAN M. HUNTER

(WITH PLATES I-IV AND TWO FIGURES)

Introduction

Abies is attacked by a great number of heteroecious rusts. In every instance it is the O, I, or aecidial phase that occurs on the fir host. Table I shows all the rusts reported for various species of *Abies*, and the hosts of the II-III stage where known.

It is quite certain that there are other species on *Abies*, the connections of which have not yet been established. This is probable for various species of *Hyalopsora*, *Milesina*, and *Pucciniastrum*.

All of the rusts of *Abies* are of the peridermial type except *Melampsora americana* and *M. Abieti-Capraearum*. As a rule the peridermia on *Abies* are very similar, in fact so nearly alike that some of them have not been and perhaps cannot be distinguished specifically, or even generically. They are readily separated, especially when fresh, into two groups on the basis of color of the spores, white and yellow. Within these groups the form of the peridermia, their location, and the effect of the rust on the host are characteristic for a few species but not for the rest. Such features of the spores as size, markings, and thickness of the walls are of help, but to a limited degree. It is of little wonder, then, that some confusion has resulted. A striking example of this is afforded by PECK's *Peridermium balsameum*. FRASER (10), in the first successful attempt to

work out the life history of *P. balsameum*, linked it up with *Uredinopsis americana* (*U. mirabilis*), but in further experimentation with the fungus from other host sources he also linked it up success-

TABLE I

RUSTS WITH 0, I STAGE ON *ABIES*

Name of rust	II, III host
1.* <i>Melampsora americana</i> Arth.	<i>Salix</i>
2.* <i>Melampsorella Caryophyllacearum</i> Schroet.	<i>Alsine</i> , <i>Arenaria</i> , <i>Cerastium</i> , <i>Malachium</i> , <i>Moechringia</i> , and <i>Stellaria</i>
3. <i>Melampsorella Symphyti</i> Bubak.	<i>Symphytum</i>
4.* <i>Pucciniastrum Abieti-Chamaenerii</i> Kleb.	<i>Epilobium angustifolium</i> , <i>E. Dodonaei</i> , and <i>E. latifolium</i>
5.* <i>Pucciniastrum Epilobii</i> (Pers.) Otth.	<i>Epilobium adenocaulon</i> , and other species of the subgenus <i>Lysimachion</i>
6. <i>Pucciniastrum Circaeae</i> (Thuem.) Speg.	<i>Circaea lutetiana</i> , etc.
7.* <i>Calyptospora Goeppertia</i> J. Kühn.	<i>Vaccinium</i> (III only)
8.* <i>Hyalopsora Aspidiotus</i> (Pk.) P. Magn.	<i>Phegopteris Dryopteris</i> and <i>P. Robertiana</i>
9. <i>Peridermium ornamentale</i> Arth.	
10.* <i>Milesina Kriegeriana</i> P. Magn.	<i>Aspidium spinulosum</i> , <i>A. spinulosum</i> var. <i>dilatatum</i> , and <i>A. Filix-mas</i>
11.* <i>Milesina marginalis</i> Faull & Watson	<i>Aspidium marginale</i>
12.* <i>Milesina polypodophila</i> (H. P. Bell) Faull.	<i>Pteris aquilina</i>
13. <i>Milesina Blechni</i> Syd.	<i>Blechnum spicant</i>
14.* <i>Uredinopsis Atkinsonii</i> P. Magn.	<i>Aspidium Thelypteris</i> and <i>Asplenium Filix-femina</i>
15.* <i>Uredinopsis Phegopteridis</i> Arth.	<i>Phegopteris Dryopteris</i>
16.* <i>Uredinopsis Osmundae</i> Magn.	<i>Osmunda Claytoniana</i> , <i>O. cinnamomea</i> , and <i>O. regalis</i>
17.* <i>Uredinopsis americana</i> Syd.	<i>Onoclea sensibilis</i> and <i>Woodwardia areolata</i>
18.* <i>Uredinopsis Struthiopteris</i> Störmer.	<i>Struthiopteris germanica</i> and <i>Woodwardia virginica</i>
19. <i>Uredinopsis Pteridis</i> D. & H.	<i>Pteris aquilina pubescens</i>
20. <i>Peridermium rugosum</i> H. S. Jackson.	
21. <i>Melampsora Abieti-Capraeacervi</i> Tubeuf	<i>Salix</i>

* Found on *Abies balsamea*.

fully with *U. Struthiopteridis*, *U. Osmundae*, *U. Atkinsonii*, and *U. Phegopteridis* (11). FAULL, WATSON, and MOSS (9) repeated these tests with like results, and then extended the investigation to species of *Milesina*. As a result they have added to the list *Milesina Kriegeriana*, *M. marginalis*, and *M. polypodophila*. Thus PECK's name (*Peridermium balsameum*) has been doing duty, not for a single species, but for many species of two distinct genera; and it probably would have continued to do so if no cultural tests had been made. No one had recognized morphological differences.

With the white spored rusts of *Abies*, as with the yellow, however, the writer has found that the spermogonia commonly show very distinctive characters. It is the spermogonia accompanying the peridermia that exhibit the most distinctive taxonomic criteria. The purpose on which this paper is based, therefore, has been to make an intensive study from this point of view. The results have been generally satisfactory so far, except for the species of *Uredinopsis* other than *U. Pteridis*.

Material and methods

The material from which the sections of *Uredinopsis Pteridis* and *Peridermium rugosum* were made was obtained from the herbarium of the department of botany, University of Toronto, and was originally collected in the western states. The rest of the material, eleven species, was obtained in the Timagami Forest Reserve of Ontario, and with the exception of four species, *Melampsora americana*, *Melampsorella Caryophyllacearum*, *Hyalopsora Aspidiotus*, and *Milesina polypodophila*, was obtained from culture experiments. These were supplemented by field collections. All of the material from the Timagami Forest Reserve was found on leaves of *Abies balsamea*.

The work of collecting and the culture experiments were directed by Professor J. H. FAULL of the University of Toronto, who was assisted by Mr. G. D. DARKER, Mr. W. R. WATSON, and Dr. E. H. MOSS. The writer wishes particularly to express her thanks to Professor FAULL for the excellent and authentic material placed at her disposal. Appreciation is also extended to Dr. H. P. BELL for some of the field material.

The diseased leaves of *Abies balsamea* used for observation were

fixed in chromo-acetic, Flemming's fluid (weaker solution), Bouin's fluid, a weak formalin solution, and Carnoy's fluid. Flemming's fluid and chromo-acetic were both found to be excellent fixatives.

The material, after being passed through the usual grades of alcohol, was cleared in chloroform or cedar oil before being imbedded in paraffin. The leaves cleared in cedar oil cut more easily than those cleared in chloroform. One lot of material of *Hyallopsora Aspidiotus* was treated in a 50 per cent aqueous solution of eau de Javelle for 3-5 hours before being passed through the different grades of alcohol. This treatment proved to have a softening effect, so that the older leaves on which this rust occurs cut better than those subjected to no special treatment, even when cleared in chloroform rather than cedar oil. After using eau de Javelle, the staining properties of the leaf seemed to be altered, so that after being stained with safranin alone the section had a yellow tinge. This, however, was not objectionable.

All material was imbedded in paraffin of 54°, 57°, or 58° C. melting point. No injury to the fungus or leaf tissue was found to occur when paraffin of a higher melting point (57°-58° C.) was used; and better sections were obtained with it, especially in the case of rusts that occur on the older leaves of *Abies balsamea*. Both longitudinal and transverse sections were made. Those studied were 4-7 μ in thickness. All drawings of spermogonia were made from transverse sections excepting in the case of *Melampsorella Caryophyllacearum*, in which the most satisfactory sections were longitudinal ones; this is because very often the spermogonia occur singly at the very tip of the leaf.

The various stains used were safranin alone, safranin in combination with Delafield's haematoxylin, light green DLI, light green NL-3, or gentian violet; also iron alum haematoxylin alone and in combination with light green DLI. The most successful results were obtained by double staining with safranin and light green DLI or light green NL-3.

A weak aqueous stain of safranin (about 1 cc. of 1 per cent solution in 45 cc. of water) proved most effective. The light green DLI used was a 2 per cent clove oil solution, while that of the light green NL-3 was a 0.25 per cent one. The stain light green DLI was one

of a lot received from Dr. CONN, Chairman of the Biological Stain Commission, Geneva, New York, to be tested. Light green NL-3 was also obtained from Dr. CONN. The sections were left in the safranin solution over night. Before staining with light green, the slides bearing the sections were carried up through aqueous alcoholic solutions and some of the safranin was extracted. Care was taken not to remove too much safranin, since light green is acid in reaction and extracts this red stain somewhat rapidly. Before counter staining with light green, the sections still retained the safranin throughout all the tissues. Not more than two or three slides at most were carried up through the alcoholic solutions at one time. After reaching absolute alcohol the slides were removed one at a time, and the sections were stained in the light green clove oil solution, which was

Description

The spermogonia described belong to the following species:

- | | |
|--|--------------------------------------|
| 1. <i>Melampsora americana</i> | 7. <i>Milesina marginalis</i> |
| 2. <i>Melampsorella Caryophyllacea-</i>
rum | 8. <i>Milesina polypodophila</i> |
| 3. <i>Pucciniastrum Epilobii</i> | 9. <i>Uredinopsis Atkinsonii</i> |
| 4. <i>Cyptospora Goepertiana</i> | 10. <i>Uredinopsis Phegopteridis</i> |
| 5. <i>Hyalopsora Aspidiotus</i> | 11. <i>Uredinopsis Osmundae</i> |
| 6. <i>Milesina Kriegeriana</i> | 12. <i>Uredinopsis Pteridis</i> |
| | 13. <i>Peridermium rugosum</i> |

applied by means of a pipette. The light green was allowed to remain from 30 seconds to a minute on the slide, after which the sections were washed in pure clove oil and the usual procedure followed.

The amount of safranin extracted before applying the light green had to be determined by experience. If too much safranin is left in, the light green is not so effective in staining; and if too much safranin is extracted, the light green stains the section so completely that one does not get good differentiation. The cuticle on the leaf is more or less indifferent to either stain, and if it retains any at all it is a faint trace of safranin. The bast of the leaf and all of the cellulose walls stain green, while the wood retains the safranin. The intermediate epidermal layer stains with safranin, while the inner epidermal cell wall stains green. All fat globules and starch bodies stain red. Chloroplastids stain either green or red, depending upon the amount of safranin extracted before the addition of the light green. The walls

of the spermatophores usually stain green, while the contents of the mycelium are influenced by the red stain. The walls at the base of the spermatophores of the spermogonium often stain red. All nuclei stain red. The mycelium found in the intercellular and air spaces is usually green. Very often the combination of the red and green stain in the fungus gives a violet effect.

1. *Melampsora americana* (fig. 1).—The spermogonia occur on balsam leaves of the current season. They are small and numerous. FRASER (10), who studied this rust under the name *M. arctica* Rostr., reports them as hypophyllous. The writer finds that this is usually true and reports only one exception. Among numerous sections only one spermogonium was found on the upper surface of the leaf. The spermogonia extend well into the mesophyll and may be raised at either side less than one-third of their height above the neighboring epidermis. The subepidermal cells below the spermogonium are somewhat collapsed. During the development of the spermogonium there is much breaking down of the host cells. Sometimes subepidermal cells at the base of the spermogonium show indications of deterioration.

It may be noted here that LUDWIG (12) states that the spermogonia of this rust are subcuticular, but after careful examination the writer finds that they are subepidermal. The epidermal cells are attacked by the fungus and are more or less broken down. The entrance of the parasite to the epidermal cells, however, is made into the inner wall and not between the cuticle and the intermediate epidermal wall, nor between the secondary layer and the inner wall of the epidermal cell. The cuticle, the secondary layer, and the outer side of the inner wall of the epidermis remain over the spermogonium, and sometimes the entire epidermal cell as in the upper right of the spermogonium shown in fig. 1. An epidermal cell at the upper left of the spermogonium is invaded by a spermatophore. The lower wall is broken down but the upper wall is intact. The cutinized parts of the guard cells remain in the upper central part of the spermogonium, and at their left is a collapsed epidermal cell. The darkened areas at the base of the spermogonium may be the remains of collapsed subepidermal cells.

The spermatophores which make up the body of the sperma-

gonium arise from a mat of interwoven mycelium at the base of the spermogonium, and are directed toward an opening in the epidermis. The spermatophores are more or less radially arranged. They have septa which mark off uninucleate cells.

The spermogonia are more or less elliptical in section, and may or may not have a central depression in the outer side. They measure 92–154 μ broad by 42–67 μ high, and average 117 μ broad by 58 μ high. The number of mature spermogonia measured was 32.

The opening through which the spermatia pass is a slit in the epidermis or an opening between the guard cells. It is usually centrally placed, but may occur a little to one side of the center. It measures 5–8 by 40–50 μ . The long direction is parallel to the long axis of the leaf.

The spermatia are small, catenulate, oblong, and measure approximately 1–1.5 by 3.2–4.5 μ . Measurements were made by the writer from prepared sections, and also by Mr. G. D. DARKER from fresh material in the field.

2. *Melampsorella Caryophyllacearum* (figs. 2–5).—The spermogonia occur on balsam leaves of the current season. They are amphiogenous, numerous, and are easily distinguishable, even without a hand lens. They appear as small, raised, round, orange spots, discrete or continuous. They are often found singly on the very tip of the leaf, but this feature is not essentially characteristic. The spermogonia are subcuticular and hemispherical, flattened in section. The mature spermogonium is characterized by a mat of closely interwoven hyphae at its base, from which the somewhat tapering spermatophores branch and extend outward toward a fairly centrally placed opening in the overlying cuticle. The material showed several stages in the development of the spermogonium.

Fig. 2 shows a very early stage. Here several strands of the septate mycelium are seen advancing through air spaces, over and between host cell walls, toward the epidermal layer. The hyphae separate the epidermal cell walls and pass between or over them and ultimately separate the cuticle from the epidermal wall. A later stage (fig. 3) shows many threads pushing along between the walls of the epidermal cells and forming a young spermogonium. There are small spaces between and above the young spermatophores. These

are filled with a substance which is probably a more or less gelatinous fluid.

A spermogonium of more advanced stage is seen in fig. 4. The tapering spermatophores advance more or less radially from enlarged basal cells toward the cuticle. Some spermatia have already formed, and are seen coming off in chains. The cuticle is unbroken and the space between it and the spermatophores is filled with a green staining substance, which is perhaps a gelatinous fluid. This fluid by pressure assists in opening the slit in the cuticle through which the spermatia emerge. In the mature spermogonium (fig. 5) the cuticle is broken and this gelatinous fluid has disappeared. The spermatophores branch from enlarged basal cells which form a mat at the base of the spermogonium. The spermatophores are septate and each cell is uninucleate. The radial arrangement of the spermatophores is not so pronounced as in the earlier stages (fig. 4). In fig. 5 the spermatophores have assumed a more erect position. Spermatia are being formed freely and are catenulate.

The spermogonia measure 99-317 μ broad by 27-59 μ high; the average measurements are 184 μ broad by 38 μ high. A total of 72 mature spermogonia was measured. ARTHUR (2) states that the spermogonia of *Melampsorella elatina* (Alb. & Schw.) Arth. (*M. Caryophyllacearum*) measure 100-130 μ broad by 40-50 μ high.

He describes the spermogonia as epiphyllous, few, and inconspicuous. This is not in agreement with the writer's findings, but ARTHUR's statement that ostiolar filaments are not present is confirmed.

DE BARY (7) describes the spermogonia as both epiphyllous and hypophyllous, but more frequently the former. He also describes tender peripheral paraphyses as a feature of the spermogonium. The opening in the cuticle through which the spermatia emerge may be a slit or a wide opening. Usually, upon breaking the cuticle flies back and leaves most of the upper surface of the spermogonium exposed. The spermatia are oblong in shape (fig. 5), and measure 1.9-3.5 by 3.9-4.9 μ .

3. *Pucciniastrum Epilobii* (fig. 6).—The spermogonia occur on leaves of the current season. They are very small, raised, subcircular, irregularly scattered, not conspicuous but easily seen with a

hand lens. A thin layer of the intermediate epidermal wall remains under the cuticle over the spermogonium. This is unlike the subcuticular condition in *Melampsorella Caryophyllacearum*, where the spermogonia are developed on very young leaves in the bud before the intermediate epidermal wall forms. The spermogonia are hypophylloous and in section appear hemispherical, flattened. The spermatophores are septate and each cell is uninucleate. They originate from a mat of mycelium at the base of the spermogonium, and are directed toward a centrally placed pore in the cuticle.

The epidermal cells at the base of the spermogonia are separated by intervening hyphae. With this exception, these epidermal cells are usually quite normal in appearance. Occasionally they may be pressed out of shape by pressure from the mycelium which separates them as it advances to form the spermogonium.

The spermogonia measure 62–137 μ broad by 15–33 μ high. The average measurements of 29 spermogonia are 84 μ broad by 20 μ high. Measurements of the spermogonia of *Pucciniastrum pustulatum* (Pers.) Diet. given by BELL (6) are 50–110 μ broad by 20–30 μ high; but as BELL made no distinction between *P. Abieti-Chamaenerii* and *P. Epilobii*, and his material was collected in the field, it is not certain with just which species of *Pucciniastrum* he worked.

The spermatia are catenulate. They are elongate and oval in shape, measuring 1.6 by 3.3 μ . Fig. 6 shows a number of spermatia in section which have been exuded from the spermogonia. The opening in the cuticle through which the spermatia are discharged is a long oval-pore measuring 13–27 by 30–75 μ . It is somewhat centrally placed. The long direction of the opening is parallel to the long axis of the leaf. Occasionally half of the upper surface of the spermogonium is exposed. One opening was found to measure 43 by 95 μ .

4. *Calyptospora Goeppertiana* (fig. 7).—The spermogonia occur on leaves of the current season. They are small and very inconspicuous, indistinctly visible with a hand lens (10 \times), very numerous, subcuticular, and hypophylloous. They are low, and hemispherical to conoidal in shape. The spermatophores arise from enlarged hyphal cells at the base of the spermogonium. They are more or less radially arranged and point toward the upper center of the spermogonium. They are septate and the cells are uninucleate. The cuticle

over the great majority of spermogonia remains unbroken, but the spermatophores are formed and the arrangement of nuclei in them is suggestive of catenulate spore formation. No bodies which can definitely be described as spermatia were found, however, even in spermogonia where the cuticle was ruptured. Sometimes the cavity above the spermatophores is filled with irregular dark staining bodies which may be degenerate spermatia.

The epidermal cells are present at the base of the spermogonium. Sometimes they are depressed. They may be fairly normal in appearance, but again they may be elongated or crushed by the hyphae which pass between them to form the spermogonium.

The spermogonia measure $42-137 \mu$ broad by $13-30 \mu$ high. The average measurements are 73μ broad by 21μ high. The number of spermogonia measured was 105. ARTHUR (4) states that the spermogonia measure $80-140 \mu$ broad by $25-30 \mu$ high. These measurements correspond somewhat closely with those just given.

ARTHUR (4) states that the spermogonia are epiphyllous and few. This is not in agreement with the writer's conclusions, after thorough examination of authentic material obtained from various culture experiments as already described. As stated, the spermogonia are hypophyllous and numerous. BELL states that he found spermogonia of *Calyptospora Goeppertia* (*C. columnaris* (A. & S.) Kühn), but he does not describe them. ADAMS (1) describes *C. columnaris* as having the stages O, I, but gives no description of the O stage.

Both Dr. E. H. Moss and Mr. W. R. WATSON, after careful examination of fresh material of infected leaves which bore both spermogonia and mature aecidia, were unable to find any spermatia. As stated previously, measurements were made of median sections of 105 spermogonia of *C. Goeppertia*. Many more spermogonia were examined which were considered immature, and among all of these only twelve were found to have a ruptured cuticle. In a few cases a mass of dark staining substance of indeterminable composition was found just above the upper center of the spermogonium, but even this seemed to be inclosed by a thin hyaline layer which may be a continuation of the cuticle.

There is evidence then that the spermogonia of this species are abortive. DODGE (8) describes vestigial or abortive spermogonia in

the pine rust, *Gallowaya pinicola* Arth. He states that host tissues above the spermogonia are not fully ruptured, and that spermatia are seldom formed. Here we have a parallel case to that of *Calyptospora Goeppertia*. In the pine rust, however, the spermogonia are few in number, while in the balsam rust they are numerous. It is interesting to note that since it has been definitely ascertained that *C. Goeppertia* bears spermogonia, the only remaining feature that separates *Thecopsora* from *Calyptospora* is that the latter lacks the uredinal stage.

5. *Hyalopsora Aspidiotus* (fig. 12).—The spermogonia occur on leaves of *Abies balsamea* of the second season. They are hypophylous, and conspicuous as round, yellow, slightly raised spots. They are subepidermal, extending from the epidermis well into the mesophyll. There is a deep mass of interwoven mycelium separating the mesophyll cells below the base of the spermogonium. This feature is much more pronounced in this species than in any of the others studied. It is characteristic of *Hyalopsora Aspidiotus*. The base of the spermogonium itself consists of a mat of enlarged hyphal threads. It is from this mat that the spermatophores which make up the body of the spermogonium proceed. The spermatophores arise singly or branch from the intertwined mycelium which forms the mat at the base of the spermogonium. They are arranged in more or less radial lines directed to a fairly central opening in the epidermis, through which the spermatia are discharged. Septa occur in the spermatophores and mark off uninucleate cells. The spermogonia in section are more or less oval or lens-shaped, and are much flattened. The spermogonia measured were 25 in number. They are very much longer than they are deep, measuring 311–496 μ broad by 86–117 μ high. They are rarely less than 390 μ broad. The average measurements are 432 μ broad by 102 μ high. These measurements correspond closely with those made by BELL. He gives the limits 400–500 μ broad by 90–120 μ deep.

MAYOR (13) describes what appear to be the spermogonia of this or a related form on *Abies pectinata*. His measurements for the spermogonia are 300–360 μ broad by 150–210 μ high. These therefore are not so broad and are deeper than those recorded by BELL or by the writer. If the mat or weft of mycelium at the base of the

spermogonium is included by MAYOR in his results, it brings his measurements nearer to ours. The spermatia are described by MAYOR as spherical, and having an average diameter of 3μ . These measurements are not in agreement with those made by the writer.

Spermatia are formed catenately (text fig. 1). They are oblong not round, comparatively large, and measure approximately $2.4-3.9$ by $5.1-8.7 \mu$. Measurements made by Mr. G. D. DARKER from fresh material obtained from culture experiments in the field are $2.4-3$ by $5.1-8.7 \mu$.



FIG. 1.—Photomicrograph of median vertical section of spermogonium of *Hyalopsora Aspidiotus*, from transverse section of leaf of *Abies balsamea*; $\times 200$.

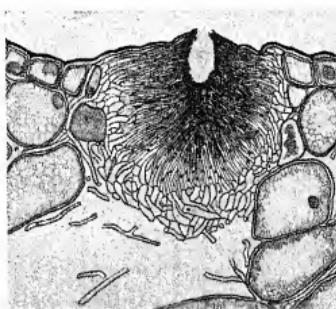


FIG. 2.—Photomicrograph of median vertical section of spermogonium of *Uredinopsis Pteridis*, from transverse section of leaf of *Abies grandis*; $\times 400$.

The spermatia escape through a somewhat centrally placed opening in the epidermis. This may be made by a break in the epidermis or by means of a stoma, the guard cells of which are always abnormal. The opening takes the form of a slit, the long direction of which is parallel to the long axis of the leaf. It may measure $4-16.7 \mu$ wide by $47-204 \mu$ long. The slit lengthens as the spermogonium matures. Text fig. 1 shows a spermogonium with numerous spermatia and the slit in the epidermis through which they are discharged.

6. *Milesina Kriegeriana* (fig. 9).—The spermogonia are found on leaves of the current season. They are inconspicuous, but can be detected by means of a hand lens as tiny spots on the blanched leaves on which they occur. They are hypophylous and occur very

abundantly. Sometimes as many as four spermogonia are found in a row in a transverse section of an infected leaf. These spermogonia occur more abundantly than in any other species of *Milesina*. They are imbedded in the mesophyll of the host leaf and are subcuticular. It is not the cuticle alone which remains over the spermogonium, however, but the intermediate granular layer of the epidermal wall immediately under the cuticle as well. The epidermal cells have disappeared, or sometimes some of them may be found crushed at the base of the spermogonium, as in fig. 9. The spermogonia rise very slightly above the neighboring epidermis. They depress the subepidermal cells beneath them. In section they are conspicuous and vary from hemispherical to subspherical in shape. Measurements made from prepared sections show a range of 84–137 μ broad by 59–84 μ high. An average of the 53 spermogonia measured is 110 μ broad by 71 μ high. Measurements made from prepared slides by Mr. WATSON are 80–140 μ broad by 60–90 μ high.

The spermatophores have septa which mark off uninucleate cells. The spermatophores arise from enlarged hyphal threads at the base of the spermogonium, and in more or less radial arrangement they advance toward a central opening in the cuticle through which the spermatia emerge. This opening takes the form of a slit, and measures 3.3–8.4 by 15–60 μ . The long direction of the slit is parallel to the long direction of the leaf.

The spermatia found in prepared sections have a range of measurements from 1.5–1.6 by 4.5–5 μ . Field measurements of spermatia made by Mr. WATSON are 1 by 4.6–7.4 μ .

7. *Milesina marginalis* (fig. 10).—The spermogonia occur on leaves of the current season. The leaves at the time of the appearance of the spermogonia are blanched. The spermogonia are inconspicuous but can be detected easily with a hand lens. They are amphigenous, but are found most frequently on the lower surface of the leaf, less frequently on the upper surface, and occasionally upon the side. The spermogonia are subcuticular, but the conditions are somewhat peculiar, as in *Milesina Kriegeriana*. The spermogonium lies under the combined cuticle and intermediate layer of the epidermal wall. The displaced epidermal cells have entirely disappeared; or sometimes, surrounded by the innermost wall, may be

found at the base of the spermogonium. The teethlike projections extending into the spermogonium from above are the remains of the granular layer which laterally separated the displaced epidermal cells from one another, and which are seen to be continuous with the intermediate layer referred to.

The spermogonium is very deeply seated in the mesophyll, and depresses the subepidermal cells beneath. Sometimes the subepidermal cells lying immediately beneath the spermogonium have collapsed. In section the spermogonia are conspicuous and vary from subspherical to almost spherical in shape. They are large, measuring 129-168 μ broad by 92-134 μ high. The average measurements of 50 spermogonia measured are 147 μ broad by 106 μ high. The ranges of measurements given by Mr. WATSON are 125-170 μ broad by 90-125 μ high.

The base of the spermogonium is made up of enlarged interwoven hyphae from which the spermatophores arise. The spermatophores are septate and each cell is uninucleate. They have a more or less radial arrangement, and are directed toward a central pore in the cuticle through which the spermatia emerge. The pore measures 1.7-5 by 20-50 μ . The long direction of this slitleike pore is parallel with the long axis of the leaf. The spermatia are catenulate. They are elongate and measure 1.6 by 5-8 μ . These measurements were made from prepared sections. Measurements made by Mr. WATSON from fresh material in the field are 1 by 4.6-9 μ .

8. *Milesina polypodophila* (fig. 11).—The spermogonia occur on leaves 2-8 years old. They are inconspicuous but can be seen distinctly with a hand lens. The affected needles are never blanched to nearly the same extent as those infected with *Milesina Kriegeriana* or *M. marginalis*. Infected portions are associated with a peculiar multiplication of twigs, producing a loose broom effect. The spermogonia are hypophylous, very deeply seated in the mesophyll, and are subepidermal. In section they are very conspicuous and are nearly spherical, large, measuring 175-234 μ broad by 175-243 μ high. BEIL gives the measurements 180-250 μ in diameter. On an average the 17 spermogonia measured by the writer are 199 μ broad by 212 μ high. The spermogonia are isolated. In the many sections examined there was only one instance where two spermogonia were found to be confluent.

There is some indication of more or less massed hyphal threads where an air space occurs below the spermogonium, but in most of the sections of *M. polypodophila* examined such a mass is entirely lacking. The base of the spermogonium itself is composed of enlarged hyphal threads interwoven to form a mat. It is from these that the septate spermatophores, more or less radially arranged, proceed to the upper middle of the spermogonium. The cells of the spermatophores are uninucleate. The spermatia are formed at the tips of the spermatophores. They are elongate and catenulate, and measure 1.7–2.5 by 5–7 μ . The spermatia are emitted through a pore which may be a slit in the epidermis or an opening through a stoma, the guard cells of which are abnormal. The opening is fairly centrally placed and measures 7–12 by 19–29 μ . The slit is not long, but its direction is parallel to the long axis of the leaf.

9. *Uredinopsis Atkinsonii* (fig. 8).—The spermogonia occur on leaves of the current season. They are hypophyllous and inconspicuous, and when mature extend somewhat deeply into the mesophyll. They are usually isolated, very seldom confluent. In section they are hemispherical and are usually quite flat on top, but may have a central depression. They are comparatively small, measuring 65–120 μ broad by 40–64 μ high. These measurements approximate those of *Milesina Kriegeriana*. On an average those of *Uredinopsis Atkinsonii* are smaller. The average measurements of 39 spermogonia are 95 μ broad by 53 μ high. They are subcuticular. The same conditions which exist in *Milesina Kriegeriana* are found in *Uredinopsis Atkinsonii*, however, namely, the combined cuticle and the intermediate layer of the outer epidermal wall remain over the spermogonium. At the upper right hand side of the spermogonium (fig. 8) the mycelium is seen separating an epidermal cell from the intermediate layer just referred to. In this figure also a spermogonium is shown in which the displaced epidermal cells have entirely disappeared. Occasionally one or two are found at the base of the spermogonium. The subepidermal cells below the spermogonium are depressed.

The spermatophores are arranged somewhat radially. They have septa which mark off uninucleate cells. The basal cells are long and tubular in shape. This is a very characteristic feature and is of great aid in distinguishing this species from *Milesina Kriegeriana*. The

opening in the cuticle through which the spermatia emerge is a lens-shaped pore or short slitlike opening which measures 4-12 μ wide by 15-25 μ long. The long direction is parallel to the long axis of the leaf.

The spermatia are small, oval, catenulate, and measure 1-3.3 by 2.5-5 μ . Measurements were made from dry and wet material as well as from sections.

The measurements of the spermogonia of *Uredinopsis* (*P. bal-sameum* Peck) given by BELL are 100-130 μ broad by 30-50 μ high. ARTHUR and KERN (5) state that the spermogonia are 100-130 μ broad by 35-50 μ high. These measurements correspond closely with those made by BELL. A short description of the spermogonia of *U. Phegopteridis* and *U. Osmundae* follows. They are so much like those of *U. Atkinsonii* that it is doubtful whether any real distinction can be recognized.

10. *Uredinopsis Phegopteridis*.—The description of the spermogonia of the species *U. Phegopteridis* is the same as that for *U. Atkinsonii*. The measurements of the spermogonia of *U. Phegopteridis* are 75-125 μ broad by 34-50 μ high. The average measurements of the 42 spermogonia measured are 95 μ broad by 48 μ high. The opening through which the spermatia emerge measures 1.6-11.6 by 15-30 μ . The spermatia measure 1.5-2 by 3.3-4.1 μ . These measurements were made from sectioned material only.

11. *Uredinopsis Osmundae*.—The description of the spermogonia of *Uredinopsis Osmundae* is similar to that of the other two species, *U. Atkinsonii* and *U. Phegopteridis*, only that the spermogonia of *U. Osmundae* were fewer in number for the number of sections cut than in the case of the others. The measurements for the spermogonia of *U. Osmundae* are 92-130 μ broad by 50-57 μ high. The average measurements are 112 μ broad by 52 μ high. This average is a little higher than that for the other two species described, but since only 8 spermogonia were measured, it is unfair to take these measurements as conclusive. The opening through which the spermatia emerge measures 3.3-20 by 15-85 μ . The spermatia measure 1.7 by 3.3 μ . Measurements were made from prepared sections.

12. *Uredinopsis Pteridis* (text fig. 2).—What is now generally considered as the O, I stage of this rust was described by ARTHUR

and KERN as *Peridermium pseudobalsameum* on materials collected by BLASDALE and HOWE from *Abies grandis*.

The spermogonia in the material sectioned were on leaves of *Abies grandis* of the second year. They are inconspicuous, numerous, hypophyllous, and subcuticular. The condition here is similar to that of *Milesina Kriegeriana*, *M. marginalis*, *Uredinopsis Atkinsonii*, *U. Phegopteridis*, and *U. Osmundae*, that is, the combined cuticle and intermediate layer of the outer epidermal wall remain over the spermogonium. The remains of epidermal cells are sometimes found at the base of the spermogonium. The spermogonia greatly depress the mesophyll tissue beneath.

The spermogonia of *U. Pteridis* are much larger than those of the other species of *Uredinopsis* described by the writer. The former measure 100-159 μ broad by 85-110 μ high. On an average they measure 127 μ broad by 98 μ high. These measurements were made from sections of the dried type material (collected by BLASDALE and HOWE, Eureka, California).

The base of the spermogonium consists of enlarged intertwined hyphal threads from which the spermatophores proceed in somewhat radial direction toward a fairly centrally placed pore in the cuticle. The spermatophores are septate, but it was impossible to find much detail because the dried tissue stained deeply. The pore in the cuticle measures 5-13.3 by 20-42 μ . The long direction of the slit is parallel to the long axis of the leaf. No clearly defined spermatia were seen, possibly because spore discharge had been completed in the material employed for histological examination.

Three other lots of material of *U. Pteridis* were sectioned and examined. Two of these were obtained from J. S. BOYCE, Washington State, and the other from H. S. JACKSON, Oregon. A summary of the measurements made from each of these follows.

(1) Spermogonia of material obtained from J. S. BOYCE measure 104-137 μ broad by 92-109 μ high. On an average they measure 121 μ broad by 102 μ high. Small bodies which are probably spermatia were found to measure 3.3 by 5 μ . The opening in the cuticle through which the spermatia emerge measures 7-17 by 20-40 μ .

(2) Spermogonia of the second lot of material obtained from the same source measure 134-154 μ broad by 67-92 μ high. On an av-

erage they measure 144 μ broad by 78 μ high. The opening in the cuticle measures 7-23 by 20-40 μ .

(3) Spermogonia of material obtained from H. S. JACKSON measure 139-184 μ broad by 75-87 μ high. On an average they measure 168 μ broad by 82 μ high. The opening in the cuticle through which the spermatia emerge measures 8-13 by 35-55 μ . In all cases this opening is a slit, the longitudinal direction of which is parallel to the long axis of the leaf.

From these measurements of the spermogonia, it will be noticed that those of the type material are somewhat vertically elongated in section, and that those of the lot listed under (1) most nearly approximate the type in measurement. The spermogonia under (2) and (3) in general are more horizontally elongated in section, and are less vertically elongated than those of the type material or of (1). These measurements indicate that the spermogonia under (2) and (3), although much larger than those given for *Peridermium balsameum*, are of that type, and stand out quite distinctly from those of the type material collected by BLASDALE and HOWE. Here the question may be raised as to whether or not all of the *U. Pteridis* material examined by the writer is authentic, or at least whether it all represents *P. pseudobalsameum*. There may be a confusion of species which have the O, I stage on *Abies grandis*.

The needles sectioned by the writer were all of the second year. In all of this material aecidia as well as spermogonia are borne. The aecidia are most certainly borne on leaves of the second year, but it is still to be determined whether or not the spermogonia appear on leaves of the first season and persist throughout the second season, or appear in the same year as the aecidia. Even though there is uncertainty as to the time of the appearance of the spermogonia in *U. Pteridis*, there is no reason for confusing it with such forms as *U. Atkinsonii*, *U. Phegopteridis*, or *U. Osmundae*, in which the spermogonia and the aecidia occur on the affected needles in the current season.

ARTHUR (4), who regards *Peridermium pseudobalsameum* Arthur & Kern as the O, I stage of *U. Pteridis*, describes the O stage of this rust, but does not state the age of the leaves on which the spermogonia occur. He gives the range of measurements of the spermogonia as 160-180 μ broad by 100-170 μ high. These measurements

are somewhat greater than those made by the writer, but they indicate that the spermogonia are vertically elongated in section. ARTHUR also states that the spermogonia are subcuticular. This is a correction of a statement made in an earlier publication (5), where it is stated that the spermogonia are subepidermal.

SCHMITZ (15) records a rust on *Abies amabilis* and one on *A. grandis*. The former case is doubtfully referred to as *P. pseudobalsameum* and in the latter identified as *P. balsameum*; the identifications were made by ARTHUR. SCHMITZ concludes that the difference between *P. pseudobalsameum* and *P. balsameum* is but slight, being merely that the former has slightly larger and thicker walled spores. No cognizance is taken of the fact that the aecidia of the former are borne on needles of the second season, and that those of the latter are borne on leaves of the current season.

WEIR and HUBERT (17) state that "The close similarity of *P. pseudobalsameum* (D. & H.) Arth. with *P. balsameum* has led us to consider them here as one species, namely, *Peridermium balsameum*." No reference in the literature previous to ARTHUR's (4) in 1925 has been made to the age of the needles on which *P. pseudobalsameum* occurs. From WEIR and HUBERT's text one would judge that they take for granted that this rust occurs on leaves of the current season, and that the name is based on a geographical factor; that is, *P. pseudobalsameum* is the name given as a rule to a rust on *Abies* in the west which corresponds to *P. balsameum* in the east.

These authors describe a rust alternating between *Abies grandis* and *Pteridium aquilinum* var. *lanuginosum* Bory (var. *pubescens* Under.), which, after comparative examination with several *Uredinopsis* species, they conclude to be *Uredinopsis Pteridis*. This rust bore aecidia on leaves of the second year, but no spermogonia were reported. No comparison of spermogonia, therefore, can be made here between this species and the type material of *P. pseudobalsameum* collected by BLASDALE and HOWE. It is fairly certain that the O, I stage of their rust is *P. pseudobalsameum*, although they assume that such is not the case. WEIR and HUBERT are in error, however, when they conclude that *P. pseudobalsameum* and *P. balsameum* may be considered as a single species, *P. balsameum*. The size and shape of the spermogonia alone demonstrate a specific distinction, even without consideration of the fact that the aecidia of *P. balsameum* are

borne on first year needles, while the aecidia of *P. pseudobalsameum* are borne on second year needles; therefore *P. pseudobalsameum* and *P. balsameum* cannot be used as synonymous terms. RHOADS, HEDGE COCK, BETHEL, and HARTLEY (14) have fallen into the same error as WEIR and HUBERT.

In their study of the life history of *U. Pteridis*, WEIR and HUBERT cultured only from *Abies* to *Pteris*. Apparently they made a guess as to the sequence of development in the opposite direction, stating that "The telial stage does not overwinter as is the common habit of such rusts. The needles of the fern must become infected during the same summer or fall in which the telia mature on the fern." The aecidia would complete their development in the following spring. They were unaware of the order of events in such a rust as *Hyalopsora Aspidiotus*, in which infection takes place in the spring on young needles, but the aecidia do not appear until the second spring after. In this latter species it happens that telia form, mature, and germinate on the fern host before the balsam needles are completely unfolded. Apparently, however, the same results would follow from overwintered teleutospores. If the teleutospores of *U. Pteridis* behave as do those of other species of *Uredinopsis* they surely overwinter, and it may be predicted that infection of *Abies* takes place when the needles are young. It is probable that there is a comparatively long period of incubation, the spermogonia developing toward the latter part of the first season and the aecidia at the beginning of the next.

13. *Peridermium rugosum*.—Some leaves of *Abies grandis* infected with *Peridermium rugosum* which were sent by H. S. JACKSON to Dr. FAULL were examined by the writer. Transverse sections 3 and 5 μ in thickness were made. The spermogonia were found to be hypophyllous, rather numerous, inconspicuous on the leaf surface, but conspicuous in section extending between the cells beneath and greatly depressing the tissues. These findings are in agreement with the description given by ARTHUR (3). In section the spermogonia are spherical rather than hemispheric or lenticular as described by ARTHUR. They are also subepidermal. The epidermal cells are often invaded by the mycelium, and the inner wall of some of the epidermal cells overlying the spermogonium may sometimes be broken down or digested. The measurements of the spermogonia are 144-

TABLE I: SUMMARY
SPERMOGONIA OF RUSTS OF *ABIES*

SPECIES	AGE OF LEAF	SHAPE (VERTICAL SECTION)	BREATH (μ)	HEIGHT (μ)	POSITION	REMARKS	SIZE OF SPERMATIA (μ)
1. Melampsora americana	Current season	Elliptical, cen- trally depressed or not	92-134	42-67	Hypophyllous	Subepidermal	1-1.5 by 3-2-4.5
2. Melampsorella <i>Caryophyllacearum</i>	Current season	Hemispherical, flattened	99-317	27-59	Ampligenous	Subcuticular	1.9-3.5 by 3.9-4.9
3. Pucciniamitrinum Epilobi	Current season	Hemispherical, flattened	62-137	15-33	Hypophyllous	Subcuticular	1.6 by 3.3
4. Calyptospora Cooperiana	Current season	Hemispherical to conoidal	42-137	13-30	Hypophyllous	Subcuticular	Aborted
5. Hyalopsora Aspidiotus	Two-three years	Lens-shaped, much flattened	311-496	86-117	Hypophyllous	Subepidermal	2.4-3.9 by 5.1-8.7
6. Milesina Kirgeriana	Current season	Hemispherical to subspherical	84-137	59-84	Hypophyllous	Subcuticular	1-1.6 by 4.5-7.4
7. Milesina marginalis	Current season	Subspherical to almost spherical	129-168	92-134	Ampligenous	Subcuticular	1-1.6 by 4.6-9
8. Milesina polyopodophila	Two-eight years	Spherical	175-234	175-243	Hypophyllous	Subepidermal	1.7-2.5 by 5-7
9. Uredinopsis Atkinsonii	Current season	Hemispherical	65-120	40-64	Hypophyllous	Subcuticular	1-3.3 by 2.5-5
10. Uredinopsis Phlegopteridis	Current season	Hemispherical	75-125	34-50	Hypophyllous	Subcuticular	1.5-2 by 3.3-4.1
11. Uredinopsis Osmundae	Current season	Hemispherical	92-130	50-57	Hypophyllous	Subcuticular	1.7 by 3.3
12. Uredinopsis Pteridis*	†	Hemispherical, vertically elongated	100-159	85-110	Hypophyllous	Subcuticular	†

* On *Abies grandis*; all others from *A. balsamea*.

† Aecidia occur in second season; time of occurrence of spermogonia unknown.

† Probably discharged when material was collected.

223μ broad by $144-200 \mu$ high. The average of the 11 spermogonia measured is 170 by 169μ . ARTHUR's (3) measurements are $140-170 \mu$ broad by $120-160 \mu$ high. These spermogonia correspond closely in their position in the leaf, and in their size, shape, and structure with those of *Milesina polypodophila* (fig. 11) found in *Abies balsamea*. Moreover, they also occur on needles older than those of the current season. The resemblance is so striking that the conclusion arrived at is that in all probability these two forms are the same species. This belief was further confirmed after critical comparative examination of the aecidiospores and the cells of the peridium. In markings and size those of *P. rugosum* closely tally with those of *Milesina polypodophila*. The spermatia of *P. rugosum* measure 2.2 by $4.5-7.6 \mu$. In size and shape they are much like those of *M. polypodophila*. Although the alternate host for *P. rugosum* has not yet been determined, it is reasonable to think that it may be found on some species of *Polypodium* in the west; and that *P. rugosum* is a species, if not identical with, at least closely related to *Milesina polypodophila*.

Summary

A summary of the preceding data is given in table I, page 21.

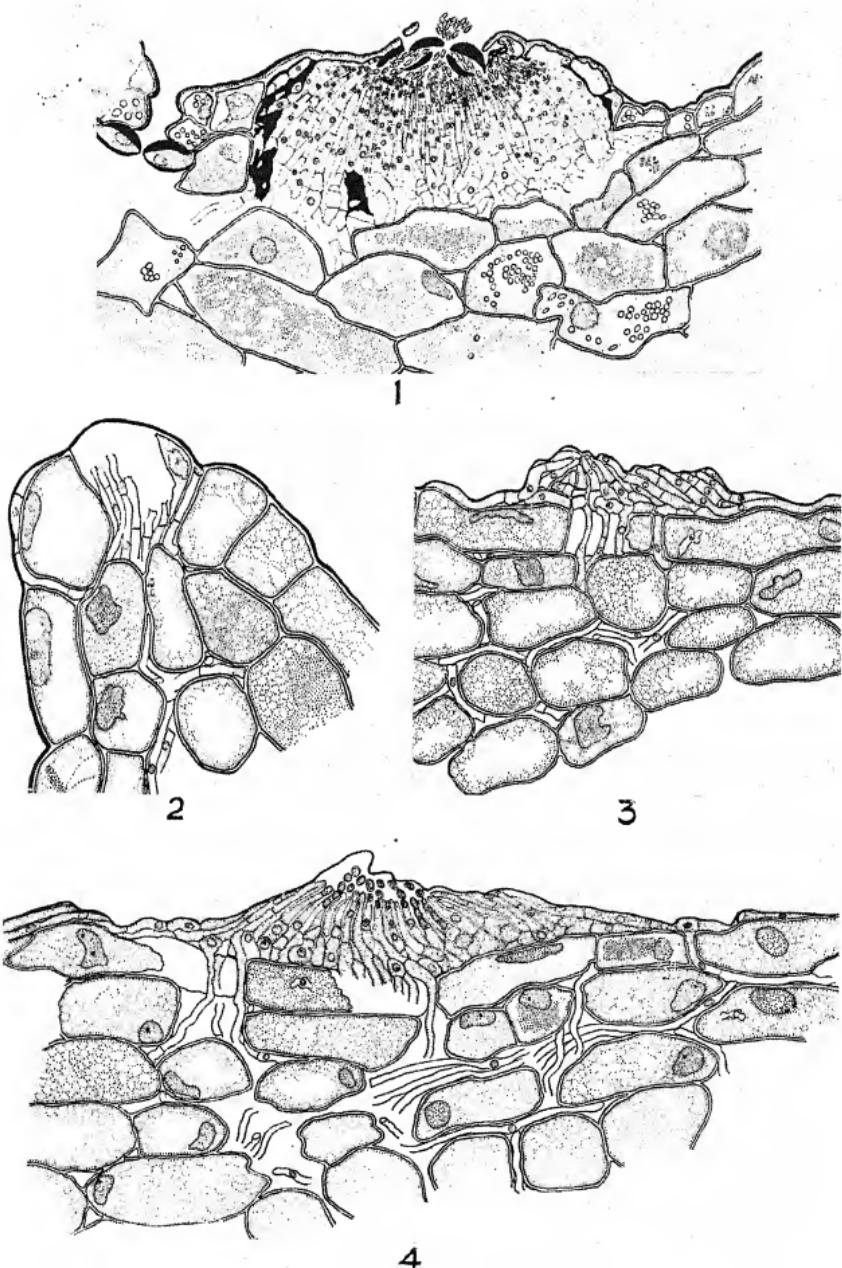
The writer wishes to express thanks to Dr. J. H. FAULL, under whose direction the work was carried on, and also wishes to express her appreciation of his contributions of material and his active interest and helpful assistance throughout the investigation.

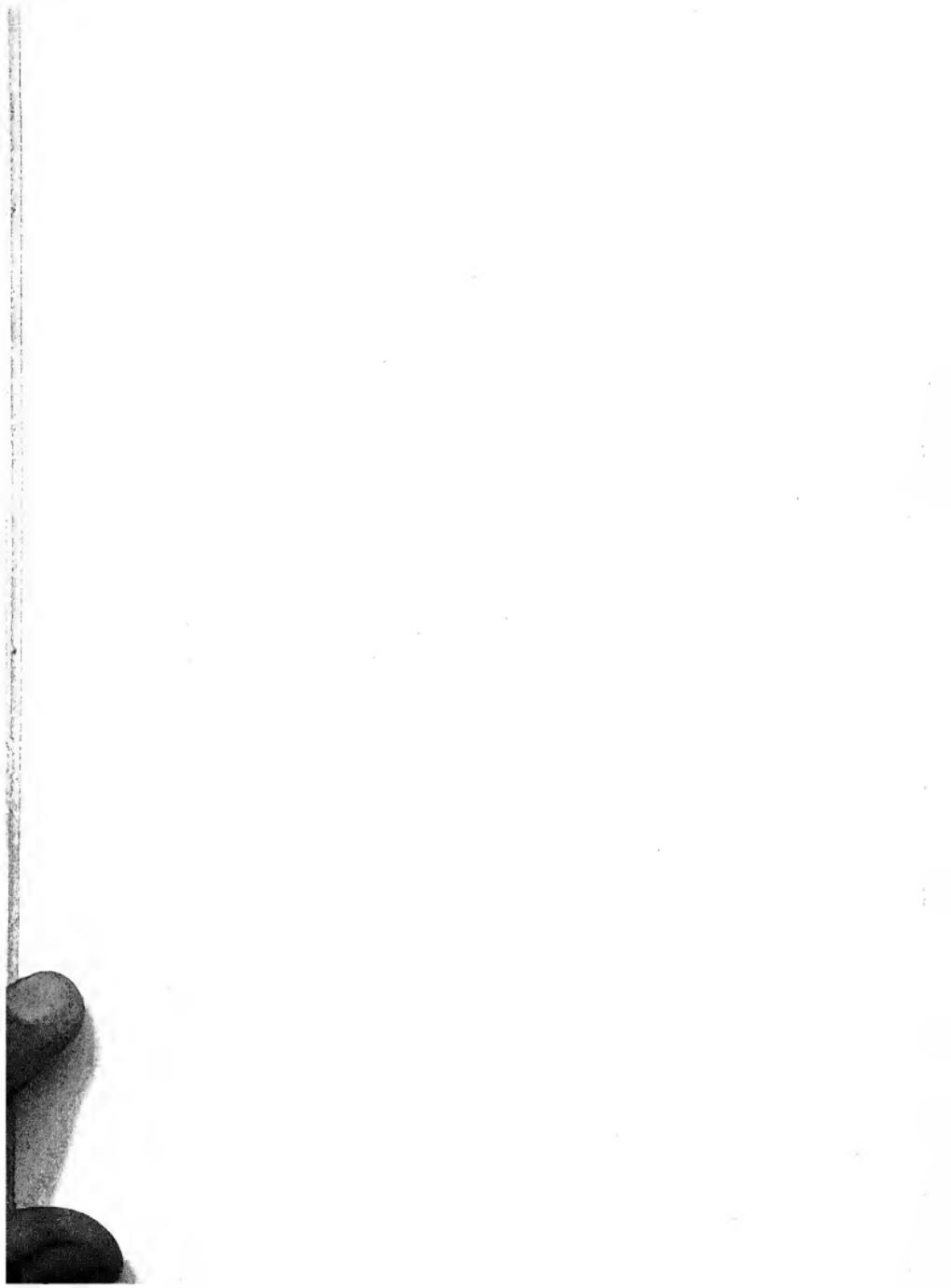
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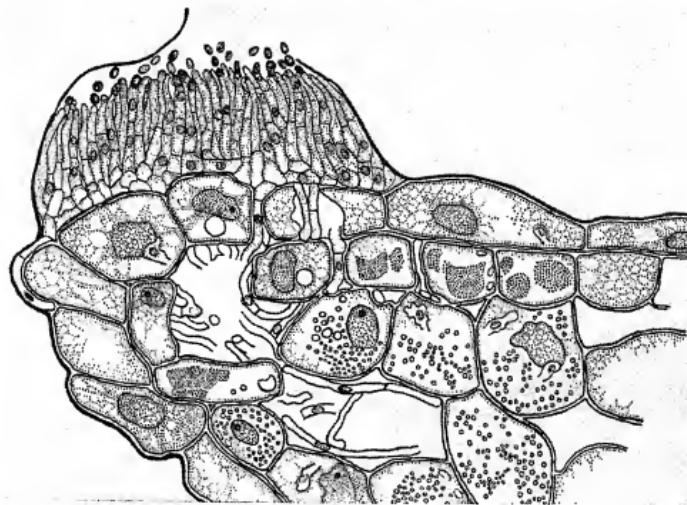
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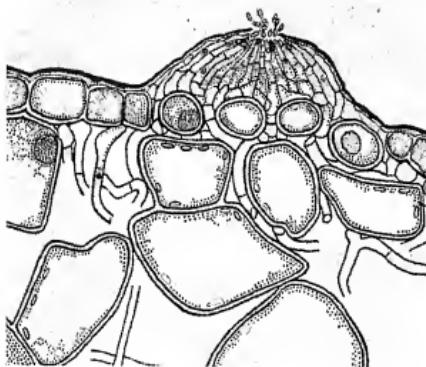
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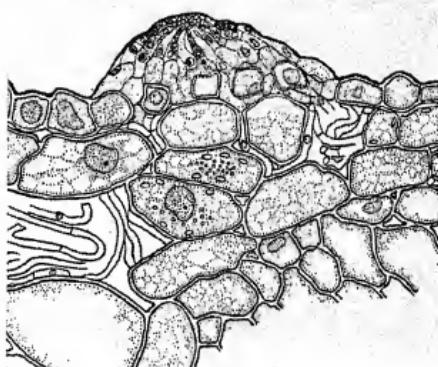




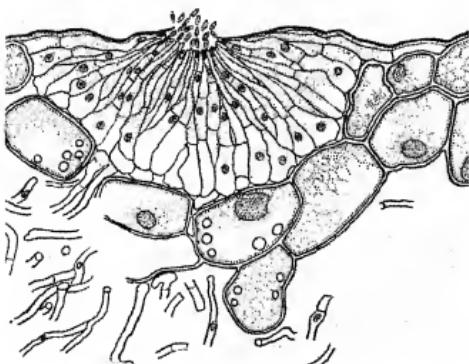
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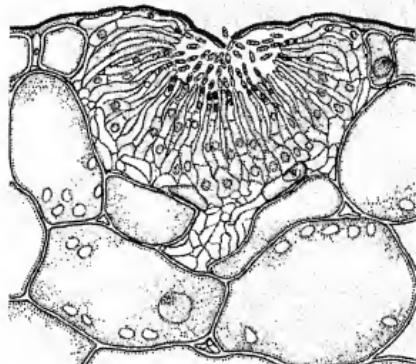
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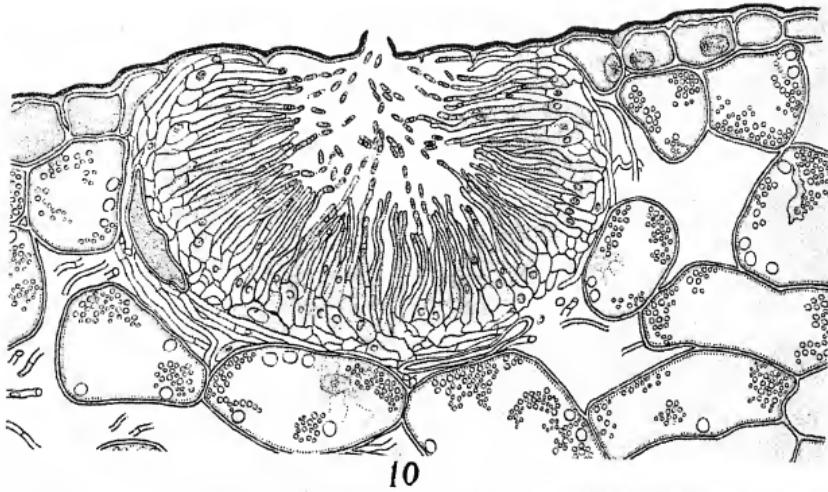


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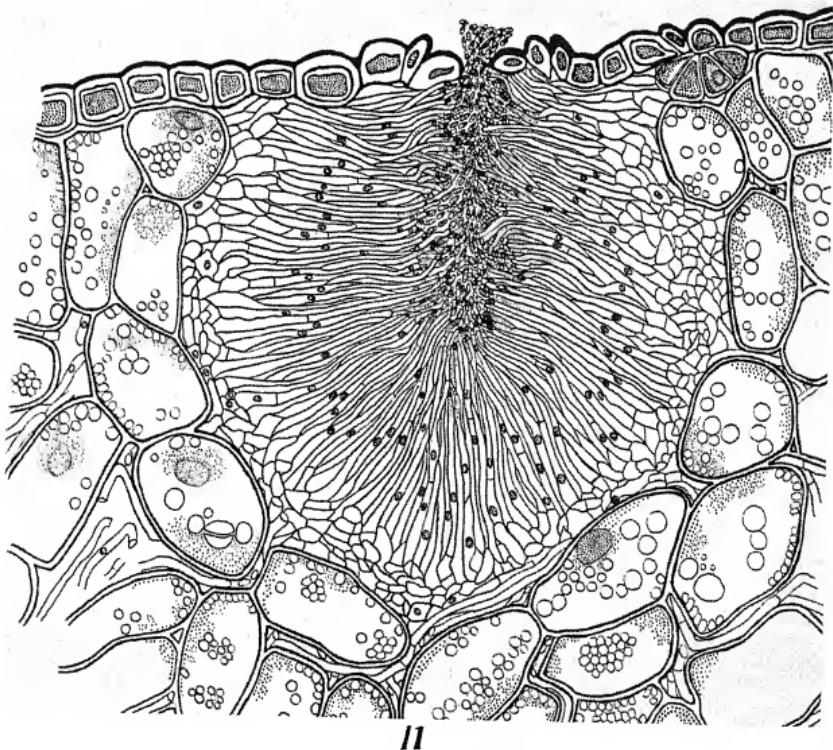


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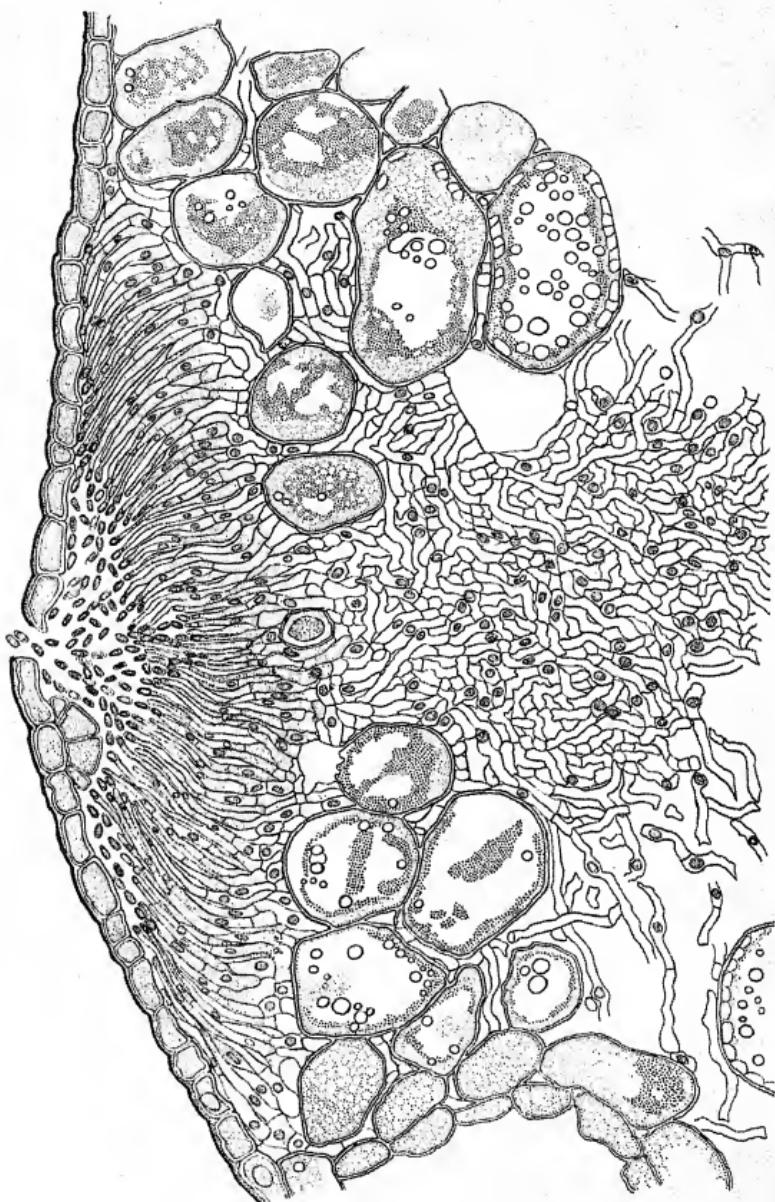


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HUNTER on RUSTS OF *ABIES*



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EXPLANATION OF PLATES I-IV

FIG. 1.—Median vertical section of spermogonium of *Melampsora americana*, from transverse section of leaf of *Abies balsamea*; $\times 413$.

FIG. 2.—Early stage in development of spermogonium of *Melampsorella Caryophyllacearum*, from longitudinal section of leaf of *Abies balsamea*; $\times 413$.

FIG. 3.—Somewhat later stage in development of spermogonium of *Melampsorella Caryophyllacearum*; $\times 413$.

FIG. 4.—Median vertical section of spermogonium of *Melampsorella Caryophyllacearum*, just before spore discharge takes place; $\times 413$.

FIG. 5.—Median vertical section of fully mature and ruptured spermogonium of *Melampsorella Caryophyllacearum*, from longitudinal section of leaf of *Abies balsamea*; $\times 413$.

FIG. 6.—Median vertical section of spermogonium of *Pucciniastrum Epilobii*, from transverse section of leaf of *Abies balsamea*; $\times 413$.

FIG. 7.—Median vertical section of spermogonium of *Calyptospora Goepertiana*, from transverse section of leaf of *Abies balsamea*; $\times 413$.

FIG. 8.—Median vertical section of spermogonium of *Uredinopsis Atkinsonii*, from transverse section of leaf of *Abies balsamea*; $\times 420$.

FIG. 9.—Median vertical section of spermogonium of *Milesina Kriegeriana*, from transverse section of leaf of *Abies balsamea*; $\times 413$.

FIG. 10.—Median vertical section of spermogonium of *Milesina marginalis*, from transverse section of leaf of *Abies balsamea*; $\times 413$.

FIG. 11.—Median vertical section of spermogonium of *Milesina polyodophila*, from transverse section of leaf of *Abies balsamea*; $\times 400$.

FIG. 12.—Median vertical section of spermogonium of *Hyalopsora Aspidiotus*, from transverse section of leaf of *Abies balsamea*; $\times 413$.

SEASONAL VARIATION IN SPECIFIC CONDUCTIVITY
OF WOOD IN TROPICAL PLANTS WITH
REFERENCE TO LEAF FALL¹

R. S. INAMDAR AND A. L. SHRIVASTAVA

(WITH SEVEN FIGURES)

Introduction and methods

The problem of leaf fall in the tropics is indissolubly connected with the general phenomenon of periodicity in plants. The complex nature of the latter phenomenon has so far eluded exact analysis of the factors concerned in the process. The investigator is at once faced with the difficulty of evaluating quantitatively the distinctive effects of internal factors in the plant and of the external environment. Is periodicity a phenomenon inherent in the living organism, irrespective of the external environment (1, 5), or is it introduced merely as an adaptation to the changing environmental factors? Even if the former view be accepted, the exact periods of rest and activity, with reference to any given set of functions, must have had some reference to the environmental factors for their origin. Cases where periods of rest and activity are changed by changing the habitat are not unknown (2, 14). On the other hand, there are plants which endeavor to retain their periods of rest and activity even in an environment where periodicity is not well marked (14). In such cases one can speak of the cumulative influence of the environment on the race being fixed in heredity as internal factors, or persisting as after effects. The organism might be plastic, in which case it will withstand the new environment with new adjustments and adaptations; otherwise it will not survive the change. The distinction between internal factors and the superficial effects of external factors is not always easy to understand. Individuals of the same species growing in an apparently similar habitat, or even different branches of the same plant, might shed their leaves at different periods of the year (14), as in *Ficus religiosa*. It would therefore be profitable to investigate the actual internal changes that take place during

¹ Contribution from the Department of Botany, Benares Hindu University.

the periods of rest and activity, instead of endeavoring to refer the phenomena to the causative internal or external factors. It is reasonable to expect some sequence in the internal changes leading to the final results, but until that sequence is well established, it is safe to speak of them as correlative changes, rather than as a sequence of cause and effect.

While the phenomenon of leaf fall in the temperate regions is commonly spoken of as a response to intense cold (12), that in the tropics is generally understood to be due to shortage of water supply (14). The effects of intense heat or insolation, however, are also to be reckoned with. The effects of each of these require careful analysis. In a place like Benares, leaf fall seems to be associated more with the driest period of the year, although this also happens to be the hottest period (April, May, and June). Trees which shed their leaves during winter are not uncommon in the area, but very few of them are entirely bare of leaves. Heat and light in this instance might be considered entirely as independent factors, or as ones that influence the phenomenon secondarily by increasing the rate of transpiratory activity, which appears to be more probable. It was therefore considered to be a matter of considerable interest to investigate whether there are any correlative internal changes in the plant during the time of leaf fall, influencing the supply of water to the transpiring leaves. With this object in view, the following experiments were conducted in Benares from April to November, 1923.

The supply of water to the leaves depends not merely on the external supply to the roots, but also on the capacity of the conducting tissues to carry water. In trees with their roots penetrating deep down in the soil, there is less likelihood of the external supply to the roots acting as a limiting factor. On the other hand, it might conceivably be argued that during the hottest period of the year the increase of temperature accelerates, if anything, the absorptive capacity of the roots, unless the soil temperature rises to a harmful extent. This latter possibility, however, is remote. If the phenomenon of leaf fall is to be associated with the supply of water to the transpiring leaves, therefore, our main consideration will have to be directed to the changes in the water conducting capacity of the vascular tissue.

A simple method to investigate the conducting capacity of the vascular tissue was devised by FARMER (4). He expresses his "specific conductivity" in terms of "specific volume," which he defines as the volume of water transmitted by a stem 15 cm. in length per sq. cm. of wood (in cross-section) under a definite pressure of water column. His method can conveniently be used for comparing the conducting capacity of the vascular tissue in different seasons of the year, on the assumption that all the water is transmitted through the wood. This method was therefore adopted in the course of the present investigation. It must be stated, however, that the specific conductivity does not necessarily bear a direct ratio to the absolute amount of water supplied to the leaves, unless another assumption is made, namely, that the water supply to the conducting elements is not limiting the rate. As already stated, this reservation does not affect the problem in the case of deeply rooted trees.

The apparatus adopted was more or less similar to the one used by FARMER (4). For the source of pure water supply for forcing water through the stem, a copper aspirator (filled with water comparatively free from dirt) was used instead of the direct supply from the water taps of the laboratory. The pressure of water column used by FARMER was equivalent to 30 cm. of mercury. This pressure could not be maintained in the present investigation, since the direct supply from the water taps contained many impurities which choked up the vessels. DIXON (3) has also found that for measuring the volume of water conducted through the stem under pressure, it is safer to adopt a lower pressure. The pressure that was given here to force the water through the stem amounted to 8.5 cm. of mercury column. This gave satisfactory results, and the readings remained constant from period to period (table I). Only those results which gave constant readings were taken into consideration.

The pieces of stem were prepared in the same way as described by FARMER. Prepared pieces were attached to the apparatus and the water was forced through them under pressure. The mean volume of water passed within a period of 15 minutes was measured as the absolute volume of water. A transverse section at the middle portion of the piece used was next taken, and the area of the wood was measured with the aid of a planimeter from camera lucida draw-

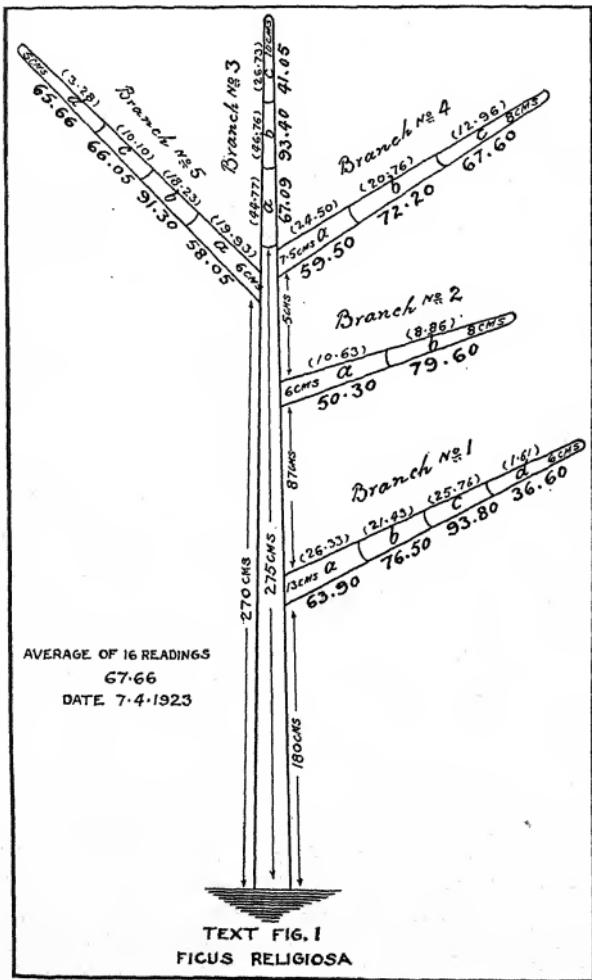
ings of the sections. The length of the piece of stem remaining constant always, the absolute volume divided by the area of the wood denoted the "specific conductivity"; or, in other words, the capacity of the wood to conduct water comparatively per given volume of the conducting elements, provided the supply of water is not limiting the rate of flow. Table I gives some preliminary readings taken from three portions (base, middle, and apex) of a branch of *Ficus religiosa*. Usually three or four readings of absolute volume were taken to ensure that the rate of flow through the stem remained constant during the period of observations.

TABLE I
PRELIMINARY READINGS WITH *FICUS RELIGIOSA*; APRIL 11, 1923

PORTION OF BRANCH	ABSOLUTE VOLUME OF INDIVIDUAL READINGS (CC.)	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY
(a) Basal.....	{ (1) 24.60 (2) 24.80 (3) 24.10	24.50	0.4147	59.50
(b) Middle....	{ (1) 21.00 (2) 20.90 (3) 20.40	20.76	0.2597	72.20
(c) Apical....	{ (1) 13.00 (2) 13.20 (3) 12.70	12.96	0.1915	67.60

Table I shows that the specific conductivity of the three portions of the branch varies considerably, a phenomenon which was also noticed by FARMER. In fact, the distinction which FARMER has drawn between trees of sympodial and monopodial habit of growth lies in the fact that in the former the specific conductivity falls off rapidly from the base of the growing shoot to the apex, while in the latter the difference is not so marked. This variation in the specific conductivity from different localities on the branch, from branch to branch placed at different levels on the tree, and from individuals to individuals, offers a serious difficulty in comparing the seasonal variations of the specific conductivity. It is not always safe to base the comparisons on averages, unless the number of observations is very great and representative of variations. For these reasons observa-

tions were made in several ways, as follows, to determine the specific conductivity comparatively in the leafy and leafless condition of the tree. Observations were made at the same time of the year



on different trees of a species, and on different branches of a tree, some having leaves still attached, others having no leaves, and still others showing newly developing leaves. Comparisons were also made between the specific conductivity of the wood from a number of observations at different seasons of the year, namely, once at the time of leaf fall, and again when new leaves were fully developed.

Preliminary results on Farmer's lines

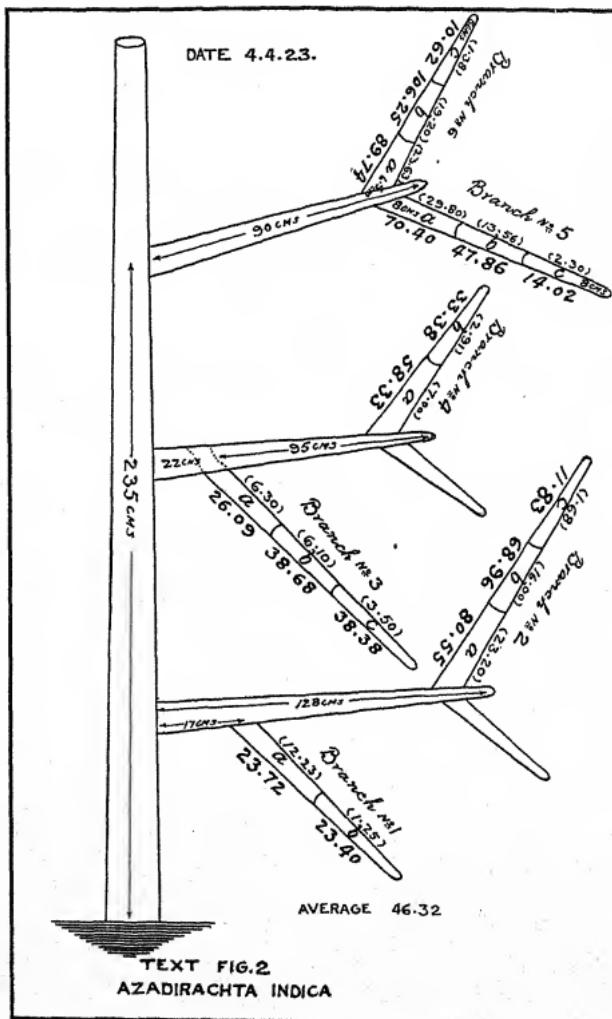
Some preliminary observations were made to confirm the distinctions drawn by FARMER between sympodial and monopodial growth, and between deciduous and evergreen trees. Although these observations have no reference to the problem of leaf fall, the results obtained are of sufficient importance to be recorded here, since no other work on similar lines appears to have been carried out on tropical plants.

DISTINCTION BETWEEN SYMPODIAL AND MONOPODIAL GROWTH

FARMER's conclusions on plants of the temperate region have already been stated. The results obtained with *Ficus religiosa* and *Azadirachta indica* are described here as representatives of monopodial and sympodial growth respectively. A number of ultimate branchlets were chosen from each tree, and the change in the specific conductivity was measured on each branch from the base to the apex. Figs. 1 and 2 indicate respectively for the two plants the relative positions on the tree of the branchlets chosen for experiment, and of the different pieces cut from each branchlet. The branchlets are numbered serially in Arabic numerals, and the pieces from each branchlet are marked *a*, *b*, etc. The figures given in brackets indicate the absolute volume of water conducted by each piece; those given in bold type without the brackets represent the specific volume or the specific conductivity. The distances are marked in cm. The results are also given in tables II and III respectively.

The results of specific conductivity included in these tables and figures show that, in the normal growing shoots of sympodial habit (branches 2, 5, 6 in table III and fig. 2), the capacity for conducting water falls off rapidly from the base to the apex. The phenomenon is not so well marked in *Ficus religiosa* with a mono-

podial habit of growth. As FARMER observes, probably one of the main causes that lead to the dormancy or death of the top bud in sympodial growth is the failure of water supply to the growing



region, thus necessitating the development of the adventitious buds. PALLADIN (11) also attributes the characteristic sympodial habit to the descending water current demonstrated by WIESNER (16). He

TABLE II
FICUS RELIGIOSA; APRIL 7, 1923

BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY
1a.....	26.33	0.4116	63.90
1b.....	21.43	0.2798	76.50
1c.....	25.76	0.2745	93.80
1d.....	01.61	0.0439	36.60
2a.....	10.63	0.2115	50.30
2b.....	08.86	0.1113	79.60
3a.....	44.77	0.6675	67.09
3b.....	45.76	0.4900	93.40
3c.....	20.73	0.5050	41.05
4a.....	24.50	0.4147	59.50
4b.....	20.76	0.2597	72.20
4c.....	12.96	0.1915	67.60
5a.....	19.93	0.3433	58.05
5b.....	18.23	0.1995	91.30
5c.....	10.10	0.1529	66.05
5d.....	03.283	0.0500	65.66
Average of readings 67.66			

TABLE III
AZADIRACHTA INDICA; APRIL 4, 1923

BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY	REMARKS
1a.....	2.23	0.0940	23.72	Branchlet from a thick and comparatively old branch; apical portion missing
1b.....	1.25	0.0531	23.40	
2a.....	23.20	0.2880	80.55	
2b.....	16.00	0.2320	68.96	
2c.....	1.68	0.1420	11.83	
3a.....	6.30	0.2414	26.00	
3b.....	6.10	0.1577	38.68	
3c.....	3.50	0.09119	38.38	
4a.....	7.00	0.1200	58.33	
4b.....	2.91	0.08717	33.38	
5a.....	29.80	0.4229	70.40	
5b.....	13.56	0.2833	47.86	
5c.....	2.30	0.1640	14.02	
6a.....	23.63	0.2633	89.74	
6b.....	19.20	0.1807	106.25	
6c.....	1.387	0.1306	10.62	
Average of readings 46.32				

observes that, due to the early development of the leaves just beneath the terminal growing point, water is withdrawn by them from the terminal bud for purposes of transpiration, resulting in the death of the terminal bud. When such plants were cultivated in an atmosphere nearly saturated with water vapor, monopodial growth was the result. That deficiency of water should influence characteristic growth is no curious phenomenon, since water is an important constituent of protoplasm, and since turgor is a condition essential to growth.

DISTINCTION BETWEEN DECIDUOUS AND EVERGREEN TREES

FARMER has drawn the conclusion that the average specific conductivity in a deciduous tree is generally higher than that in an

TABLE IV
MANGIFERA INDICA; AUGUST 30, 1923

BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY
1a.....	13.73	0.2665	57.57
1b.....	9.83	0.2192	44.84
1c.....	2.45	0.0772	31.73
2a.....	13.60	0.1900	71.57
2b.....	5.30	0.1624	32.62
2c.....	1.116	0.0828	13.40
3a.....	14.65	0.2576	56.08
3b.....	6.03	0.2005	30.07
3c.....	0.683	0.0816	8.33
4a.....	7.90	0.2080	38.00
5a.....	18.83	0.3402	55.34
5b.....	7.90	0.2142	36.90
6a.....	6.20	0.1322	46.74
6b.....	1.50	0.0733	20.46
Average of readings 38.83			

evergreen. Although his observations, except in those cases where he has worked with the deciduous and evergreen species of the same genus, do not necessarily indicate that a deciduous tree growing under similar conditions of habitat indicates always a higher specific conductivity than an evergreen, he has been led to this general conclusion from a great number of observations made on the two types of plants. It was a matter of interest to investigate whether the differences noted by him were connected primarily with the relative

demands on the water supply made by the two kinds of plants. The results obtained on an evergreen, *Mangifera indica* (table IV), were therefore compared with those of a few deciduous trees, the leaves of which varied in their transpiring capacity, inducing thus a variation in their demands on the water supply. As deciduous trees *Ficus*

TABLE V
PSIDIUM GUAVA TREE NO. I

DATE	BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY
June 2, 1923	1a.....	5.96	0.2553	23.34
	1b.....	4.55	0.1932	23.56
	1c.....	2.25	0.1168	19.26
	1d.....	0.45	0.0834	5.40
	2a.....	3.21	0.1908	16.82
	2b.....	2.80	0.1189	23.54
	2c.....	1.25	0.0645	19.38
	3a.....	6.96	0.2663	26.13
	3b.....	4.11	0.1964	20.92
	3c.....	2.16	0.1100	19.63
	4a.....	3.03	0.1701	17.81
	4b.....	2.00	0.1130	17.70
	4c.....	1.00	0.0591	16.95
	Average of readings 19.26			
September 26, 1923	1a.....	3.00	0.1633	18.37
	1b.....	2.46	0.1437	17.05
	1c.....	1.48	0.1082	13.67
	2a.....	1.85	0.1645	10.94
	2b.....	1.08	0.0846	12.80
	2c.....	0.3525	0.0634	5.55
	3a.....	7.06	0.2576	27.01
	3b.....	4.56	0.2109	21.10
	3c.....	1.27	0.0863	14.71
	3d.....	0.385	0.0443	8.69
	4a.....	3.73	0.2224	16.77
	4b.....	3.73	0.1710	21.63
	4c.....	3.00	0.1740	17.24
	4d.....	0.40	0.0563	8.00
	Average of readings 15.25			

religiosa (table II), *Azadirachta indica* (table III), and *Psidium guava* (table V) were used in the order of their transpiring capacities. The tables include a number of observations on different branches, and the averages are used for comparison.

It will be seen that the evergreen *Mangifera* has a lower average specific conductivity (38.83) than the deciduous *Ficus religiosa*

(67.66) or *Azadirachta indica* (46.32), but the specific conductivity of the deciduous *Psidium guava* (average varying from 15.25 to 19.26) is actually lower than that of the evergreen *Mangifera indica*. It may be noticed further that the variations in the specific conductivities of the deciduous *Ficus religiosa* and *Azadirachta indica* are in the same direction as their relative transpiring capacities. It may safely be concluded that the distinctions drawn by FARMER between the evergreens and deciduous trees relate primarily to the demand on the water supply made by the leaves for purposes of transpiration. In general, an evergreen may be expected to be more "xerophytic" in construction with reference to transpiration than a deciduous tree, with the consequent result that the specific conductivity in the former is in general lower than that in the latter.

Seasonal variations in specific conductivity with reference to leaf fall

OBSERVATIONS AT ABOUT THE SAME TIME OF YEAR DURING LEAF FALL

The variations in the specific conductivity of the wood with reference to leaf fall were tested in several ways. In the first series of observations, comparisons were made at about the same period of the year between individuals of the same species, and branches of the same tree, showing variations from the leafy to the leafless condition, some also showing development of new leaves in the latter condition. The species used for this series of observations was *Bauhinia variegata*, which sheds its leaves about the end of April, and remains in a leafless condition for only a short period of about one month at the most. In the first instance the averages from a number of results obtained on trees in different conditions are compared. Tree no. 1 contained only old leaves, tree no. 2 old and young leaves mixed together, and tree no. 4 only young leaves. The results are summarized in tables VI, VII, and VIII^a respectively. The averages of specific conductivity show a regular gradation according to age, from 61.02 in tree no. 1 to 57.58 in tree no. 2 and 50.12 in tree no. 4. The specific conductivity reaches its maximum value just before leaf fall, and suddenly drops down immediately after leaf fall, especially when the young leaves come out as fully developed structures.

TABLE VI
BAUHINIA VARIEGATA TREE NO. 1, APRIL 17, 1923

TWIG NO.	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY
1.....	5.43	0.2251	24.20
2.....	22.15	0.3300	67.10
3.....	16.06	0.1909	84.00
4.....	8.33	0.1887	44.15
5.....	12.26	0.1769	69.30
6.....	12.30	0.2183	56.30
7.....	11.65	0.2195	53.10
8.....	11.03	0.1896	59.80
9.....	14.30	0.2025	70.60
10.....	12.76	0.2079	60.00
11.....	6.63	0.1246	53.20
12.....	13.13	0.1671	78.50
13.....	14.46	0.2231	64.80
14.....	14.65	0.1918	76.30
15.....	11.80	0.1628	72.24
16.....	5.80	0.1533	37.80
17.....	11.23	0.1473	76.20
18.....	4.83	0.0949	50.90
Average of readings 61.02			

TABLE VII
BAUHINIA VARIEGATA TREE NO. 2

DATE	BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC CONDUCTIVITY OR SPECIFIC VOLUME
May 7, 1923	1a.....	7.40	0.1129	65.50
	1b.....	6.93	0.1073	64.50
	2a.....	7.50	0.1186	63.20
	3a.....	6.90	0.1201	57.40
	3b.....	9.50	0.1254	75.70
	4a.....	7.00	0.1500	46.60
Average 62.15				
May 22, 1923	1a.....	12.76	0.2060	59.50
	1b.....	7.70	0.1360	56.60
	2a.....	14.33	0.2278	62.90
	2b.....	9.36	0.2071	45.19
	3a.....	6.95	0.1917	36.50
	3b.....	12.03	0.1695	70.90
Average 56.58				
	4	1.80	0.0358	50.20
	5	1.71	0.0366	46.50
Average 48.35				
Average of readings 57.58				

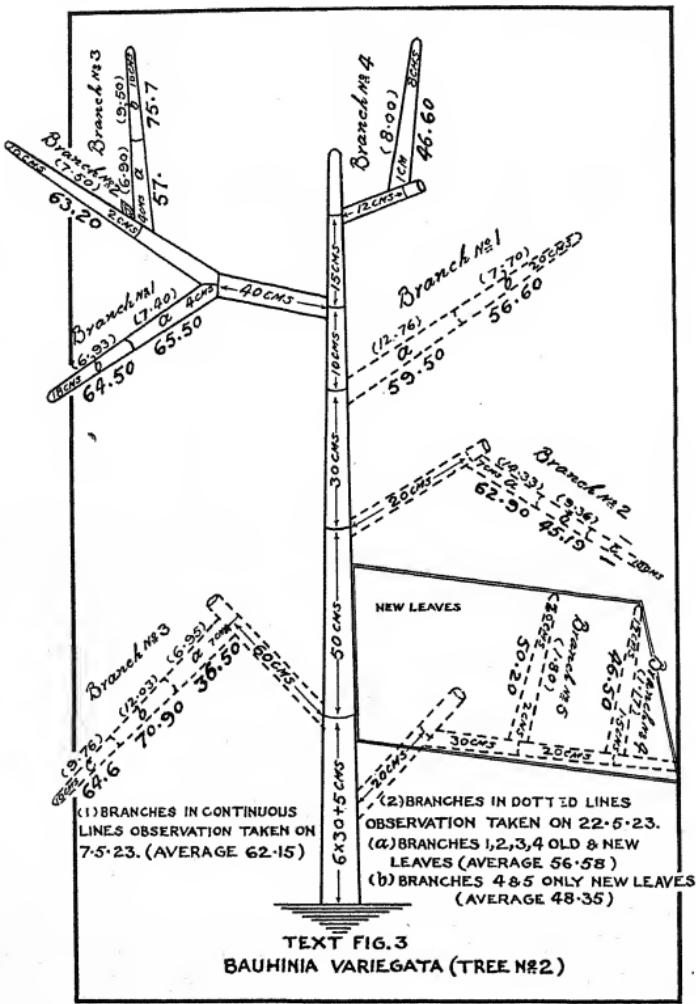
In the second set, detailed observations were made on tree no. 2 on different branches in different stages of growth. The observations on May 7, 1923 were made on branches which contained a large proportion of old leaves and a few young leaves; and those on

TABLE VIII
BAUHINIA VARIEGATA TREE NO. 4

DATE	BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC CONDUCTIVITY OR SPECIFIC VOLUME
May 10, 1923	1a.....	12.50	0.2260	55.30
	1b.....	10.90	0.1782	61.10
	2a.....	3.23	0.0973	33.40
	3a.....	13.23	0.2479	53.30
	3b.....	12.10	0.2050	59.00
	4a.....	6.30	0.1260	50.00
	4b.....	3.76	0.0772	48.70
	5a.....	8.00	0.1530	52.10
	5b.....	6.86	0.1186	59.00
	6a.....	4.76	0.0997	47.70
	6b.....	2.31	0.0593	40.00
	7a.....	3.51	0.0837	42.00
Average of readings 50.13				
October 28, 1923	1a.....	6.50	0.1402	46.30
	1b.....	4.55	0.1019	44.65
	1c.....	1.65	0.0526	31.30
	2a.....	9.90	0.2115	46.80
	2b.....	7.40	0.1370	54.01
	2c.....	3.275	0.0637	51.40
	2d.....	0.7625	0.04349	17.50
	3a.....	7.35	0.1757	41.97
	3b.....	4.075	0.1130	36.60
	4a.....	5.275	0.1363	38.70
	4b.....	4.30	0.0997	43.10
	4c.....	0.675	0.07307	9.20
	5a.....	8.00	0.1664	42.67
	5b.....	2.90	0.1213	23.90
Average of readings 35.71				

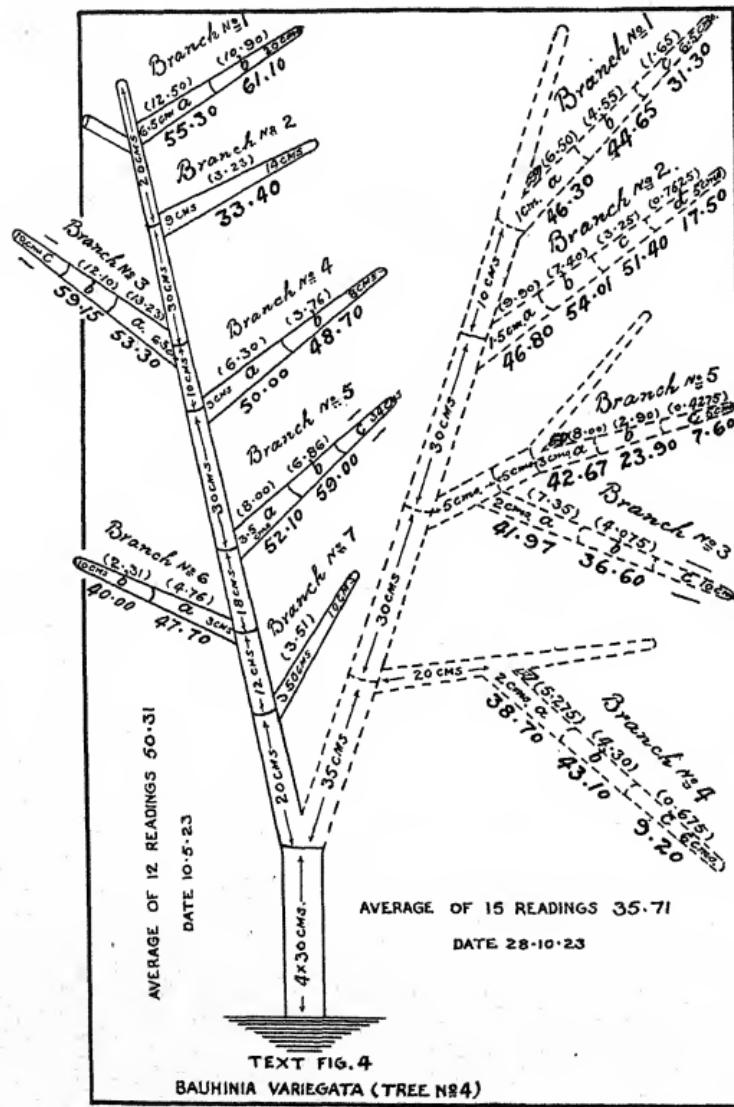
May 22 included branches, some of which contained a still larger proportion of young leaves, and others only the young leaves. The detailed results are given in fig. 3, where the letterings have the same significance as in the previous figures. On comparing the averages from these three lots (62.1, 56.58, and 48.35 respectively), it will be seen that the gradation of specific conductivity with reference to

age is similar to that noticed in the first set of observations, indicating again a sudden fall immediately after the new leaves are produced.

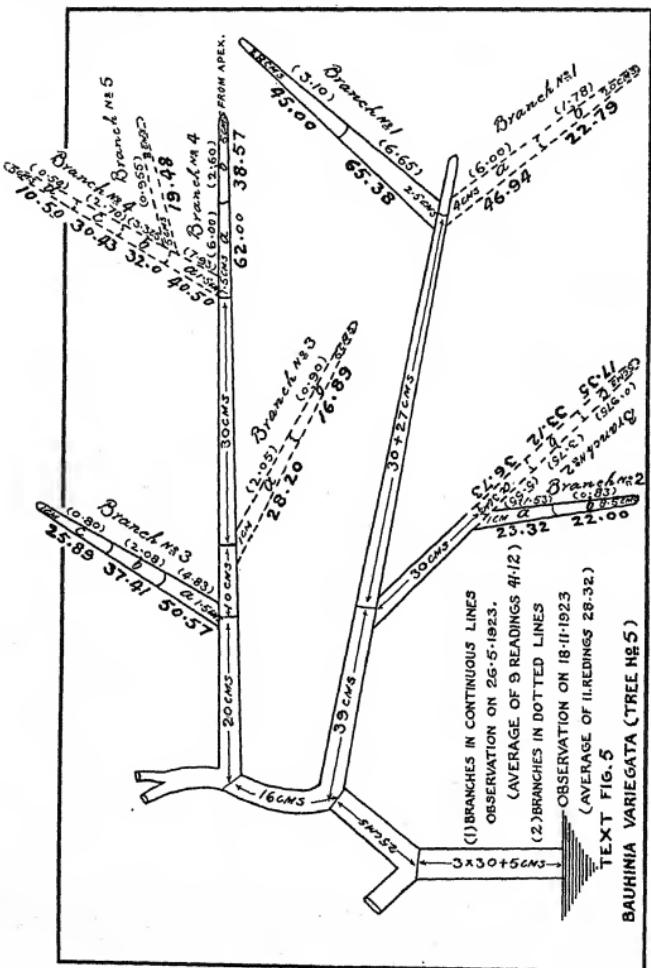


OBSERVATIONS AT DIFFERENT PERIODS OF THE YEAR

For this series of observations, comparisons were again made between the averages of individual trees and of different branches of the



same tree at different periods of the year, that is, once during the period of leaf fall and again when the new leaves were fully developed. In the first set of observations, the averages of results of trees



nos. 1 and 2 which were obtained in May 1923 (tables VI and VII) are compared with those of tree no. 4 (table VIIIb) which were obtained on October 28 when the new leaves were fully developed. The averages of trees nos. 1 and 2 are 61.02 and 57.58 respectively, but that of tree no. 4 is much lower, 35.71, thus confirming again the general conclusion already drawn. It is also worth noticing here

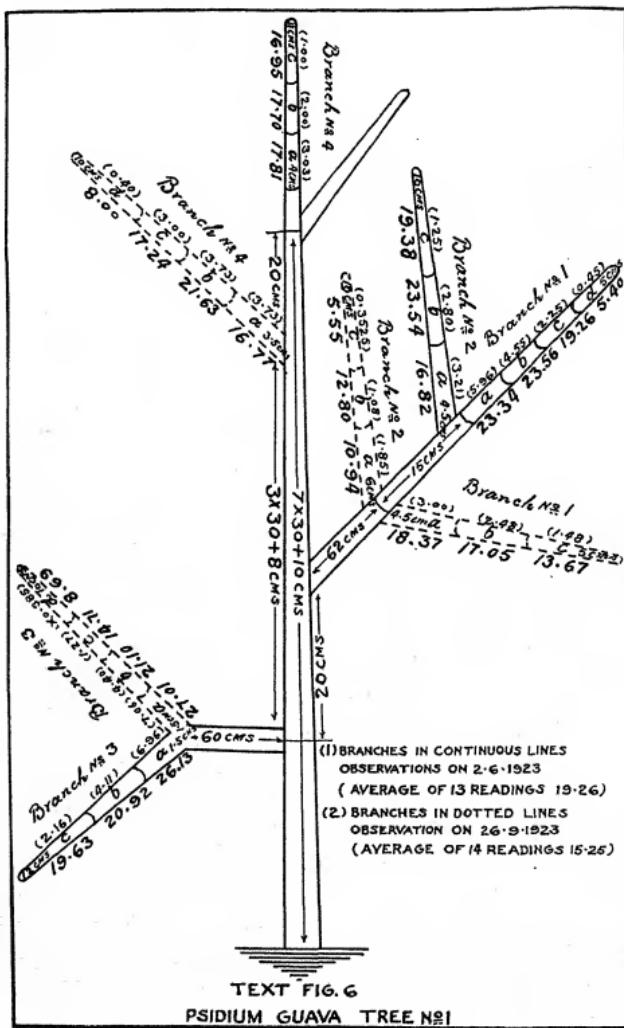
TABLE IX
BAUHINIA VARIEGATA TREE NO. 5

DATE	BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY
May 26, 1923	1a.....	6.65	0.1017	65.38
	1b.....	3.10	0.0689	45.00
	2a.....	1.53	0.0656	23.32
	2b.....	0.85	0.0388	22.00
	3a.....	4.83	0.0955	50.57
	3b.....	2.08	0.0566	37.41
	3c.....	0.80	0.0309	25.80
	4a.....	6.00	0.0968	62.00
	4b.....	2.60	0.0674	38.57
	Average of readings 41.12			
November 18, 1923	1a.....	6.00	0.1278	46.94
	1b.....	1.78	0.0790	22.79
	2a.....	5.55	0.1511	36.73
	2b.....	3.75	0.1132	33.12
	2c.....	0.975	0.0562	17.35
	3a.....	2.05	0.0727	28.20
	3b.....	0.90	0.0533	16.89
	4a.....	7.93	0.1745	45.50
	4b.....	3.325	0.1041	32.00
	4c.....	2.70	0.0887	30.43
	4d.....	0.50	0.0476	10.50
	5a.....	0.955	0.0485	19.48
Average of readings 28.32				

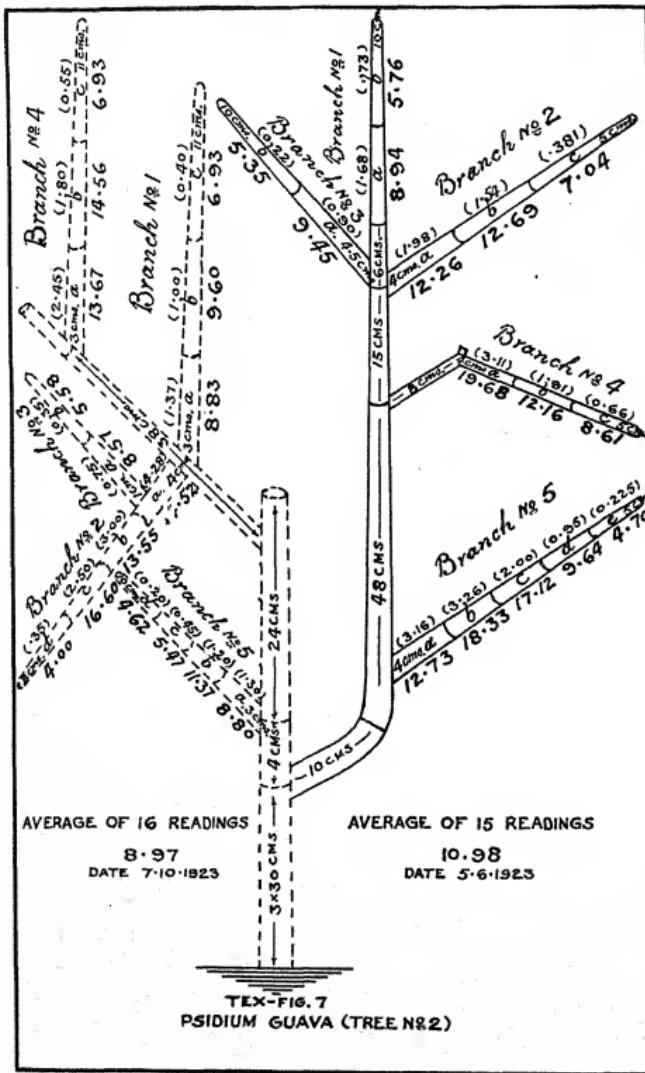
that the transpiring capacity of the atmosphere is much lower in October than in the summer months of April, May, and June, a point which will be referred to later.

The most convincing proof of the general phenomenon comes from the second set of observations, where specific conductivities of different branches of the same tree are compared in different seasons of the year. For this set of observations *Psidium guava* was included along with *Bauhinia variegata*. In *B. variegata* two trees (nos. 4 and

5) were used. In each of these the first set of observations was made on a number of branches in May 1923, when the plants were just producing new leaves, and the second set in October and November,



when the new leaves were fully mature. The results are given in diagrammatic form in figs. 4 and 5, and are also tabulated in tables VIII and IX respectively. Similar observations were made on two



trees of *Psidium guava*, the first set of observations in each tree falling in the month of June when the trees were entirely leafless, and the second set in September and October when the new leaves

TABLE X
PSIDIUM GUAVA TREE NO. 2

DATE	BRANCH NO. AND PORTION OF BRANCH	MEAN ABSOLUTE VOLUME (CC.)	ACTUAL AREA OF WOOD (SQ. CM.)	SPECIFIC VOLUME OR SPECIFIC CONDUCTIVITY
June 5, 1923	1a.....	1.68	0.1878	8.94
	1b.....	0.73	0.1266	5.76
	2a.....	1.98	0.1615	12.26
	2b.....	1.54	0.1213	12.69
	2c.....	0.381	0.0541	7.04
	3a.....	0.90	0.0952	9.45
	3b.....	0.23	0.0411	5.67
	4a.....	3.11	0.1580	19.68
	4b.....	1.91	0.1559	12.16
	4c.....	0.66	0.0766	8.61
	5a.....	3.16	0.2484	12.73
	5b.....	3.26	0.1778	18.33
	5c.....	2.00	0.1168	17.12
	5d.....	0.95	0.0985	9.64
	5e.....	0.225	0.0479	4.70
Average of readings 10.98				
October 7, 1923	1a.....	1.375	0.1550	8.83
	1b.....	1.00	0.1041	9.60
	1c.....	0.40	0.0576	6.93
	2a.....	4.28	0.2948	14.52
	2b.....	3.00	0.2213	13.55
	2c.....	2.50	0.1500	16.60
	2d.....	0.35	0.0920	4.00
	3a.....	0.75	0.0875	8.57
	3b.....	0.35	0.0621	5.58
	4a.....	2.45	0.1792	13.67
	4b.....	1.80	0.1236	14.56
	4c.....	0.55	0.0793	6.93
	5a.....	1.30	0.1479	8.80
	5b.....	1.20	0.1055	11.37
	5c.....	0.45	0.0822	5.47
	5d.....	0.20	0.0433	4.62
Average of readings 8.97				

had developed well. The results of *Psidium guava* are represented in figs. 6 and 7 and in tables V and X respectively, which do not require any further explanation. The results are mutually confirmatory. The averages during the earlier part of the year are always higher than those during the latter part. Thus for instance, in *Bauhi-*

nia variegata, tree no. 5 (cf. fig. 5 and table IX), the average of nine readings during the period the new leaves were just being produced is 41.12, while it is 28.32 for twelve readings at the time when new leaves were fully mature.

Discussion and conclusions.

The mutually confirmatory results from different sets of comparisons leave no reason for doubting that the capacity of the conducting elements for conducting water is highest just before or during the time of leaf fall, and decreases when the new leaves have come. From the data in hand, a continuous graph of seasonal variations in specific conductivity throughout the year cannot be drawn unless more observations during different periods of the year are taken. The general conclusion can be drawn, however, that variations in the specific conductivity appear to be correlated with the varying demands on the water supply made by the transpiring leaves. The conditions for transpiration are very severe during the summer months of April, May, and June, when the shedding of leaves generally occurs in Benares. The rainy season sets in during the last week of June and continues until the middle of October. The cold period commences immediately afterwards, that is, the first week of November, and continues up to about the middle of February or the beginning of March, when spring sets in, to be followed later by the intense dry heat of the summer months. The increased humidity during the rainy season and the low temperature during the cold season retard transpiration, and specific conductivity is comparatively low during these periods. It begins to rise during the summer months with the increase in the rate of transpiration. That the specific conductivity is correlated with the varying demands made by the leaves on the water supply is also confirmed by FARMER's and our observations on the deciduous and evergreen trees. This correlation is well seen in a further set of observations on the relation between the specific conductivity and the structure of the wood embodied in a separate paper (10). An inspection of the table included there indicates that, in general, the specific conductivity decreases with the "xerophytic character" of the leaves. The greatest value is found in a creeper, *Ipomea pentaphylla*. FARMER has also noticed an increase in the conductivity of the climbing species as compared with the

creeping or shrubby forms. In the case of the climbers, the transpiratory stream has to traverse a long distance, and the cross-sectional area available for transportation being comparatively small, the large demand on the top is met by increasing the specific conductivity (6).

That there should be a correlation between the demands made by the leaves on the water supply and the specific conductivity is evident from HOLMES' (7) and RIVETT'S (13) observations on the constitution of wood from the standpoint of its efficiency for conducting water. Variations in the specific conductivity are determined by the proportion of efficient water conducting elements to the total quantity of wood, their cross-sectional area, and their length, the last influencing the number of walls the water has to traverse. A reference to the table in the publication referred to (10) also indicates that the average area of the vessels varies in general with the specific conductivity, with few exceptions. In other words, variation in the specific conductivity is merely an expression of variation in the anatomical construction of the wood according to the demands made by the plants on water supply. HABERLANDT gives a great number of illustrations showing the correlation between the anatomical construction of the wood and the physiological requirements of the plants with reference to water. JOST also has shown that the extent of the transpiring surface influences the differentiation of the vascular system in a remarkable manner. Likewise KOHL'S and SCHENCK'S experiments on a number of plants grown in damp and dry atmosphere indicate that the development of the vascular system is necessarily correlated with the physiological necessities of the plants for water supply.¹

The fact that variations in the specific conductivity are correlated with the needs of transpiration is also remarkable in another way. The phenomenon appears to be another expression of the general principle of LE CHETALIER'S theorem mentioned by Mrs. SHREVE (15) in her recent paper on the seasonal variation of transpiring power in *Encelia farinosa*. According to LE CHETALIER'S theorem, if a system is subjected to stress it tends to move in such a direction as to counteract the effects of the stress. An increased demand on the water supply is met by the plant by a simultaneous

¹ JOST, KOHL, and SCHENCK, quoted from HABERLANDT 6.

increase in the capacity of the wood to conduct water, inducing an absolute increase in the supply that may reasonably be postulated, provided the supply of water to the conducting elements is not limiting the rate of flow. This general principle appears to be capable of a wider application with reference to the internal working of the living organism than merely to water supply. The problem is discussed by the first writer in a recent publication under the heading "Auto-regulation of physiological processes" (9), to which only a passing reference is necessary here.

The explanation of the depression in the specific conductivity when new leaves are produced probably lies in the fact that new growth takes place in the wood. This new growth consists of many living elements and comparatively undeveloped tracheids and vessels, reducing thus the efficiency of the conducting channels.

From the foregoing description it is clear that there is, during the time of leaf fall, an increased supply of water to the leaves which can safely be postulated by the increased capacity of the wood for conducting water, provided the water supply to the conducting elements is not limiting the rate of flow. If water supply has anything to do with the phenomenon of leaf fall, therefore, the water lost by the leaves by transpiration during the period must be still greater, inducing an unfavorable balance between supply and demand. This point can be determined only when the relations between the supply of water and the demands made by the transpiring leaves are thoroughly investigated during different seasons of the year. In measuring transpiration, one has to take into consideration not merely the effect of external environment on the process, but also variations in the internal transpiring power of the plant, which Mrs. SHREVE has clearly demonstrated (15). Work on these lines is in progress; meanwhile it is sufficient to record here the correlation existing between the period of leaf fall and the increase in the conducting capacity of the wood.

Summary

1. There is a fundamental distinction between monopodial and sympodial growth, the conductivity in the latter falling off rapidly from the base to the apex.

2. The distinction drawn by FARMER between deciduous and evergreen trees refers primarily to the respective demands made by them on the water supply for purposes of transpiration.

3. There is a correlation between the period of leaf fall and the increased capacity of the wood for conducting water.

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STRUCTURE OF SPORE WALL IN GANODERMA

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(WITH PLATE V)

In the classification of the Polyporaceae, spore characters have usually received but little attention. This is due largely to the fact that in many forms spores have not as yet been observed. An incorrect interpretation of spore structure in certain forms which have been variously placed in the genera *Fomes*, *Ganoderma*, and *Elvingia*, however, has partially been responsible for the failure to use what is undoubtedly a valuable diagnostic character. The present investigation has been undertaken in an attempt to clear up the misconceptions which have arisen in this regard.

The genus *Ganoderma* was established by KARSTEN (7) as including only one species, *G. lucidum*, characterized by having the pileus covered by a shiny crust, and the spores ovate or elliptical, warty, and yellowish brown in color. Subsequent systematic writers, like PATOUILlard (10) and MURRILL (9), have retained the genus *Ganoderma* but on the basis of the shiny crust alone. Others, like SACCARDO (11), have given it only subgeneric rank. The warty character of the spore coat has been considered by them all as of subordinate importance. PATOUILlard, it is true, has used this character in subdividing his genus *Ganoderma*, but the other writers have noted it only in specific descriptions. In fact, MURRILL places many of the forms which show quite definite affinities with *G. lucidum* as to spore characters in KARSTEN's genus *Elvingia*, which indicates the small importance he attaches to such characters.

All of the writers just cited, as well as others, as a matter of fact have misinterpreted the character of the spore wall. This was first pointed out by ATKINSON (2, 3) in two papers which appeared in 1908. He showed that the outer surface of the spore wall in a number of forms examined by him is smooth, not echinulate or verrucose as had been stated previously. In the first of the two papers he states:

The spore wall is hyaline or nearly so and is perforated by numerous slender rod-like extensions of a brown or yellowish brown substance which appear as

if they were projections of the colored contents of the spore. These do not extend beyond the outer surface of the wall, and they radiate from the endospore through the hyaline wall. They are especially prominent at the smaller end of the oval spore where the hyaline wall is considerably thicker, sometimes forming a broad cone-like cap to the spore.

In his second paper, dealing with *Ganoderma appplanatum* Pers., he states:

Not only is the spore wall hyaline or nearly so, it appears to be of a less firm consistency than the dark rods or perforating substance. In some cases, perhaps due to a certain age of the spore wall, when its consistency is less firm than at other times, the spore wall collapses to a certain extent and there is a tendency for the hyaline part of the wall to collapse between these dark areas, thus giving a roughened or slightly echinulate appearance to the spore.

In another part of the same paper he states, "The hyaline or nearly hyaline wall is perforated with numerous short dark lines or plugs which radiate from the spore content or endospore membrane through the episporule and end even with its external surface."

It will be seen that ATKINSON was in doubt as to the real nature of the "dark rods" to which he ascribed the echinulate appearance in surface views of the spore. It is interesting in this connection to note that PATOUILLARD in 1899 had observed that the spore wall in the forms studied by him was differentiated into distinct episporule and endospore. He states:

Elles sont formées d'une membrane interne épaisse et colorée en brune ou jaune plus ou moins foncé. Sur cette membrane on observe souvent des petites verrues serrées; l'épisporule est mince, incolore et se moule exactement sur les asperités de l'endospore, c'est elle qui est échancree et forme une pointe incolore à la base de la spore.

MISS AMES (1), working in ATKINSON's laboratory, has confirmed ATKINSON's description of the spore wall structure. She lists sixteen species of *Ganoderma* examined, and states that they are all characterized by having spore walls smooth, with dark lines extending into the hyaline or nearly hyaline wall from the darker spore content.

WHITE (15) gives a decidedly different interpretation to the structure of the spore wall in *Fomes appplanatus*. He states:

In studying its development we find that the basidiospore starts out with a hyaline wall and then that later within this outer thin walled basidiospore a

rough-coated thick and yellow walled endospore is formed. The spore "wall" in one sense then is accordingly double. As the endospore is more shortly elliptical than the original basidiospore, the tip of the latter is not occupied, and this hyaline tip being thin walled and without supporting contents usually collapses. This gives to the mature spore the "truncated" appearance so invariably noted.

Finally, BULLER (4) disagrees with WHITE's interpretation, and in general confirms ATKINSON's observations. He states:

It seems to me that each spore has a continuous rather thick wall made up of two layers: (1) an outer very thin, colorless, homogeneous layer formed whilst the spore is growing from a tiny rudiment to full size, and (2) an inner and a much thicker layer formed more slowly during the ripening of the spore, of a whitish or yellowish color and marked from within outwards by numerous fine yellowish brown striae. What the nature of these striae is I do not know, but I do not regard them as papillae.

From these citations it will be seen that the structure of the spore wall in these interesting forms is still a matter of uncertainty. This is hardly to be wondered at when we consider that apparently all the observations so far have been made on intact spores, either in collected spore material or in hand sections of the sporophore unstained in any way. When we remember that the spore wall is scarcely 0.5μ thick, the difficulty of making definite observations will be understood. When, therefore, Dr. FAULL suggested to me the advisability of making a more thorough study of the subject, I gladly availed myself of the opportunity so generously furnished by him. He placed at my disposal excellent material of a number of forms. To this has been added a huge mass of spore material of *Ganoderma (Fomes) appplanatum* kindly given me by Dr. J. H. WHITE.

Material and methods

The basis of this study has been material of *Ganoderma appplanatum* and *G. tsugae*; of the former I have had abundant spore material as well as sporophore material fixed in picro-sublimate; of the latter I have had good wet material in alcohol. In addition to this I have studied wet material of *G. tornatum* and *G. Lionettii*, spore material and dry sporophore material of *G. lobatum*. For comparison, material of a distinctly different form, *Fomes fomentarius*, was given to me by Dr. FAULL both as imbedded and spore material, and has been included in the study. Miss AMES included this form in the

genus *Ganoderma* on the ground of spore characters. As a matter of fact the spores are of a very different type, so much so that it seems impossible that she could have observed them at all. Previous microscopic studies of *Ganoderma lucidum* had verified the conclusions of ATKINSON (2) so far as the similarity of the spore wall in this form to that in the other forms mentioned is concerned.

As the spore coat is hard, the material for critical study was imbedded in paraffin of 60° melting point, and for most of the sectioning resort was had to the cooling action of an ether spray, a freezing attachment for the microtome not being available. As the spores of *G. applanatum* average about 5 μ in thickness, those of *G. tsugae* being somewhat larger, sections were cut down to 2 μ in thickness.

In trials to obtain a differential staining of what seemed to be two parts of the spore wall, the best results were obtained by the use of safranin and Licht Grün. Each of these if used separately was found to stain both parts of the spore wall, the endospore taking up the stain more strongly. A beautiful differential staining, however, was obtained when the sections were kept in the safranin bath (1 per cent in 50 per cent alcohol) from 12 to 24 hours, run quickly up through the various grades of alcohol, and kept in the Licht Grün bath (0.5 per cent in 90 per cent alcohol) for from 10 seconds to 1 minute, and then rapidly dehydrated and passed into xylol. This was used as the regular method of staining throughout the investigation.

Mature spores

In thin sections of the sporophore mature spores could be found in abundance, and many of them could be studied in section. *G. tsugae* proved on the whole to be more satisfactory, as its spores are larger than those of *G. applanatum*. The microscopic examination was made with a Zeiss apochromatic 1.5 mm. objective with compensation ocular no. 8. Higher power oculars (Zeiss nos. 12 and 18) were tried, but it was found that they did not give a satisfactory definition.

The microscopic picture, as found in the case of many hundreds of spores examined, showed quite definitely that the interpretation of ATKINSON, Miss AMES, and BULLER is incorrect, while that of

WHITE in the main represents the actual structure of the wall. The hyaline episporule with its conspicuous thickening at the narrower distal end takes on a beautiful green color, the Licht Grün having removed all the safranin in this area. On the other hand, the thicker endospore retains the safranin very strongly where the counter staining had not been too prolonged. The spore content, for the most part, takes on a much lighter stain than the endospore, the nucleus being visible as a more deeply stained mass in many of the sections. From the endospore project fine processes or spines, which are somewhat thicker at the inner end out into the episporule. These projections appear in some cases not to extend quite to the outer surface of the episporule, although I am not prepared to speak definitely on this point. These projections or spines show a red stain uniform with that of the endospore, and appear quite definitely as outgrowths from it. They are not rodlike extensions of the spore contents, as ATKINSON and Miss AMES believed, nor are they striae extending through the endospore wall as indicated by BULLER. These projections are frequently to be found, especially in *G. tsugae*, beautifully defined in the hyaline cap or beak. Here they show as definite red lines against the greenly stained groundwork of the beak.

A further consideration of this hyaline cap or beak is necessary. WHITE pictures it as empty. He uses the term "not occupied," and accounts for its collapse, which is so frequently to be seen in mature spores, on this supposition. The whole beak, however, even in the thinnest sections, is stained uniformly green, which would not be the case of course were there a space between the endospore and the episporule in this region.

Figs. 1 and 3 show the structure of the spore wall in *G. tsugae* and *G. applanatum* respectively, the endospore being marked in black and the spore contents being omitted. In addition to the points just mentioned, fig. 1 brings out another interesting feature of the spore wall, which ATKINSON has already noted, but which the microphotographs accompanying his papers do not illustrate; that is, the fact that at the proximal end of the spore there is a small pore in the endospore which is somewhat asymmetrically placed. Around this pore the endospore projects outward, almost if not

quite to the outer surface of the episporé. This, as ATKINSON stated, marks the point of attachment of the spore to the sterigma. The view expressed in most systematic works on these forms, that the spore is attached to the sterigma by its hyaline or (as is frequently stated) truncate narrower end, has been disproved by ATKINSON, WHITE, and BULLER.

Figs. 2, 4, and 5 give similar sectional views of the spores of *G. lobatum*, *G. tornatum*, and *G. Lionettii* respectively. The structure of the spore wall in these forms is seen to be similar in all essential respects to that found in *G. tsugae* and *G. appplanatum*. In the case of the last two forms, however, there were no projections of the endospore out into the hyaline beak, although the beak itself takes the Licht Grün stain even more strongly than in the other forms.

It may perhaps be wondered how such able observers as ATKINSON and BULLER could have misinterpreted the structure of the spore wall. It is quite explicable when we consider that the endospore, although yellowish brown in color, is quite transparent. Thus when the microscope is focused in the median plane of the spore, the projections above and beneath appear in the picture even under the highest magnifications and give the optical effect of striations. Certainty as to the structure can be attained only after a study of many very thin spore sections, something which I believe neither ATKINSON nor BULLER made.

Reference has already been made to the observations of ATKINSON on the collapse or shrinkage of the hyaline episporé, while, as WHITE has noted, the collapse of the hyaline beak in *G. appplanatum* is a phenomenon very generally observed. As a matter of fact, in all dry material most mature spores show this collapse so markedly that in systematic works the spores of these forms are generally described as "truncate at the base." In the case of two of the forms studied, *G. tornatum* and *G. Lionettii*, where the long hyaline beak is not supported by any projections of the endospore, the beak apparently does not collapse, but instead breaks off. As to the collapse at other regions of the spore wall, I have not seen any evidence of the episporé becoming molded on to the echinulate surface of the endospore, as described by PATOUILLARD and indicated by ATKINSON. Commonly, however, the episporé seems to have shrunk so as

to have its outer surface resting on the ends of the spines of the endospore. The variation in thickness of the episporic is no doubt due to the presence of varying quantities of water. This question will be discussed later in dealing with the chemical nature of the spore wall.

Development

BULLER has already indicated the general course of development of the spore wall. He states that the endospore begins to make its appearance only during the ripening of the spore, that is, after the spore has attained its full size. A careful study of developmental stages in *G. appplanatum* has shown that, while there is no sign of endospore formation until after the spore has taken on its definitive shape, measurements indicate that the spore continues to increase in size after this process has begun. Figs. 7-13 show stages in spore development with special reference to the differentiation of the spore wall. In the stage illustrated in fig. 7, the young half-grown spores were found stained almost entirely green after the safranin Licht Grün treatment. A few coarse granules were to be found scattered irregularly in the cytoplasm, which had retained the safranin stain, but these bore no demonstrable relationship to the cell wall. In this stage the nuclei had not yet migrated from the basidium.

Fig. 8, showing in section two basidiospores, illustrates what must be considered as the earliest demonstrable stage of endospore formation. Here we have the episporic and cytoplasm stained green. In addition there is a line of rather coarse granules which have retained the red stain, and which appear just inside the spore surface. At the apex of the spore, they have been laid down at a distance from the point. Between them and the surface is a distinct area free from granules which represents the episporic of the mature spore. A similar stage is illustrated in figs. 9 and 11, where a spore is shown in longitudinal and transverse section respectively.

In figs. 10 and 12 a later stage is shown. The granules have fused into a continuous membrane, from which short projections have grown out toward the episporic surface. It seems highly probable that up to this stage, and in fact even later, the limiting membrane of the spore has not yet assumed the character of a definite wall, although there has been a differentiation of a hyaline layer. The outgrowth of spiny projections from the endospore, which finally

reach almost or quite to the outer surface of the spore, would be difficult to understand if a definite episporule had already been formed. On the other hand, the presence of a plastic episporic area bounded by an outer limiting membrane would allow for this outgrowth without any difficulty. WHITE's conception of an endospore quite separate from the episporule is incorrect, I think. It is true that spores and fragments of spores can frequently be found which show portions of the episporule separated from the endospore, but this is probably a tearing along the line of demarcation between differently organized layers of a single wall, rather than a separation of two distinct walls.

Chemical composition

To verify more fully the conclusions reached as a result of microscopic examination, and to ascertain if possible the chemical nature of the episporic and endosporic layers, I subjected the wall to microchemical tests. The work of GILSON (5), WINTERSTEIN (16), and VAN WISSELINGH (13) has shown that the cell wall in most fungi does not contain cellulose. VAN WISSELINGH has further shown that its place is taken for the most part by chitin. Repeated and prolonged tests made on the spores and sections of sporophores of *G. applanatum*, *G. tsugae*, and *G. tornatum* with chlorzinciodine, iodine in potassium iodide with dilute H_2SO_4 , and cuprammonium oxide gave no evidence of cellulose, either in the spore wall or in the mycelium of the sporophore. Both chlorzinciodine and cuprammonium oxide produced a distinct swelling of the episporule, but it did not lead to its separation from the endospore layer. HANSEN (6), in his study of the spores of *Coprinus stercorarius*, has recorded a similar swelling of the episporule in that form, when the spores were treated with chlorzinciodine, which in the case of that form led to the separation of the two layers.

Tests for chitin were carried out according to VAN WISSELINGH'S method and VOUK'S (14) modification. Of the two, VAN WISSELINGH'S original method was found much more satisfactory. It consists in heating the material to be examined in concentrated KOH in a sealed tube to a temperature of 160°–180° C., which changes the chitin to chitosan, washing with 90 per cent alcohol, and then testing on the slide with dilute iodine in potassium iodide to which a drop of dilute H_2SO_4 is added. The chitosan gives a very character-

istic reddish violet color reaction when treated in this way. Dry spore material of *G. appplanatum*, celloidin sections of the same species, and hand sections of *G. tsugae* were used for the purpose. After heating, the KOH solution was diluted with distilled water to enable centrifuging, the material separated in a centrifuge, and washed with 90 per cent alcohol. The spores and shreds of sections remaining after this treatment were then treated on the slide. It was found in the case of spore material that, in the final centrifuging with alcohol, the whole mass distributed itself on the sides of the tube and stuck there as if it were gummed, and could not be dislodged by the centrifuging process.

An examination of the spore material after treatment with the coloring reagent showed both episporule and endospore intact. The cell contents had entirely disappeared, of course. The episporule remained quite hyaline, while the endospore had taken a beautiful reddish violet color. Figs. 13 and 14 show two spores thus treated in optical section, the blackened portion of the wall representing the reddish violet endospore. As will be noted in comparing these figures with fig. 3, there has been a slight swelling of both episporule and endospore, with a shortening of the spiny processes on the latter, so as to separate them distinctly from the surface of the spore. The results obtained in this test show that ATKINSON's conception of the spore wall is erroneous, and that the striae observed by BULLER are an optical illusion.

It appears certain that the endospore does not consist entirely of chitin. According to VAN WISSELINGH, chitosan dissolves in dilute HCl, and an attempt to dissolve out the endospore after treatment with KOH failed even when the slide was heated. We must conclude, therefore, that the endospore consists of chitin and some other compound or compounds at present unknown.

The study of the episporule was more difficult and puzzling. I was inclined to believe that this portion of the wall contains a gum or slime. The presence of such a substance seemed probable from the fact that spores caught on sporettraps made of microscopic slides adhere very firmly, and can be washed off only with difficulty. The episporule, however, could not consist entirely or even mainly of a gum, as it does not swell up very appreciably when the spores are

kept in water even for long periods. I have not been able to obtain fresh spores for examination, however, and they might conceivably give somewhat different results from spores which have been kept for several years on a glass slide.

I attempted to obtain a differential stain of the episporule with Ruthenium red, which according to MANGIN (8) is an extremely valuable differentiating stain for pectin bodies and the gums and slimes derived from them. While a stain of any kind is admittedly a very unsafe means of distinguishing groups of chemical compounds, it was thought that the use of this one might throw light on the question of the composition of the episporule. Spores of *G. applanatum* stained with Ruthenium red (1 part in 5000) undoubtedly give a color in the episporic region, but the endospore and also the spore contents take up the stain. It is obvious therefore that this is not a specific stain for these bodies; in fact, TOBLER (12) has already shown that other bodies, such as glycogen, take up the stain strongly.

Results from treating sections of the sporophore of *G. tornatum* were even less satisfactory. The very pronounced hyaline beak of the spore remained unstained, while the endospore and spore content became colored. The hymenial layer also appeared red, while the brown trama remained unstained. The presence of glycogen would account for the staining of the hymenial layer and the spore contents, but not for that of the endospore.

Finally I attempted to dissolve the episporule, leaving the endospore intact. For the purpose, following VAN WISSELINGH, spore material of *G. applanatum* was heated in glycerin to 290° C. (the boiling point of glycerin). In this way evidence was obtained of the solution of the episporule, but portions of it usually remained. Heating in dilute H₂SO₄ gave much better results. Spore material of both *G. applanatum* and *G. tornatum* heated on the slide in from 5 to 20 per cent H₂SO₄ gave large numbers in which the episporule had entirely disappeared, leaving the endospore intact. This reaction indicates that the episporule has the characteristics of a hemicellulose, although it gives no color reaction with chlorzinciodine or with iodine and dilute H₂SO₄. In any case its chemical composition is obviously very distinct from that of the endosporic layer of the spore wall.

Conclusions

My conception of the spore wall structure in these forms is as follows. The epispore represents the primitive spore wall, and is probably comparable with the undifferentiated spore wall of such a form as *Fomes fomentarius*, which is very thin, and which, treated by the same methods used for *Ganoderma* spores, has shown no differentiation whatever. It consists of a hemicellulose with possibly a gum, which latter, if present, functions in attaching the spore to the surface upon which it falls. The endospore is composed of chitin and other compound or compounds. It is laid down on the inner margin of the epispore as a series of granules which later fuse to form a membrane. This thickens and develops on its outer surface spiny processes which project into the epispore at a time when the latter is still plastic. The whole endospore structure obviously functions as a sort of skeletal support to the thin and collapsible primary spore wall.

It seems probable that this highly specialized structure has to do with preserving the spore through unfavorable seasons. It is a fact that the spores of both *G. appplanatum* and *G. lucidum* are extremely difficult to germinate under laboratory conditions. WHITE, in his work on the former species, was rarely able to obtain germination, and could not establish the factors which governed it. My work on *G. lucidum* was even less successful, for, although attempted repeatedly, I have never succeeded in obtaining a single germination. If we take a thin walled spore such as that of *Fomes fomentarius*, germination of fresh material is readily to be obtained according to FAULL, who has made a thorough study of the subject.

Possibly the fact that *G. appplanatum*, and presumably other species of *Ganoderma*, discharge spores over long periods during which conditions for germination cannot always be favorable, while *Fomes fomentarius* discharges only during a comparatively short period in the spring, may bear some relation to the differing structure of the spore wall.

The peculiar structure of the spore wall described in this paper is characteristic of species found in such widely separated areas as America, Europe, and India. It would seem to be a character of much greater importance from a systematic standpoint than many

of those at present being used in the classification of the Polyporaceae. A revision of the genera *Fomes*, *Ganoderma*, and *Elvingia*, which in standard systematic works are given as containing species with the spore characters here described, together with other forms showing quite different spore characters, seems to be urgently required if our classification is to represent real relationships.

As indicated by the title of this paper, I consider that all forms showing the spore characters described should be brought together under the genus *Ganoderma* Karst.

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EXPLANATION OF PLATE V

All figures have been drawn with the aid of an Abbé camera lucida, using Zeiss apochromatic 1.5 mm. objective, N. ap. 1.30 mm. and compensating ocular no. 8. They represent a magnification of approximately 2000 diameters.

FIG. 1.—Basidiospore of *Ganoderma tsugae* in longitudinal section.

FIG. 2.—Basidiospore of *G. lobatum* in longitudinal section.

FIG. 3.—Basidiospore of *G. applanatum* in longitudinal section.

FIG. 4.—Basidiospore of *G. tornatum* in longitudinal section.

FIG. 5.—Basidiospore of *G. Lionettii* in longitudinal section.

FIG. 6.—Basidium of *G. applanatum* showing first signs of basidiospore formation on sterigma to left; section fixed in picrosublimate and stained with safranin and Licht Grün (same process used for figs. 7-12).

FIG. 7.—Longitudinal section of basidium of *G. applanatum*, showing three basidiospores about half developed; larger dots represent granules stained red, rest of the spore cytoplasm being stained green.

FIG. 8.—Longitudinal section of basidium of *G. applanatum*, showing basidiospores which have assumed their definitive shape but not their full size; first signs of endospore shown as a line of granules stained red inside the epispor.

FIG. 9.—Longitudinal section of basidiospore of *G. applanatum* which has become detached from the sterigma, showing about same stage as in fig. 8.

FIG. 10.—Longitudinal section of basidiospore of *G. applanatum*, showing endospore granules fused into continuous wall and spiny projections beginning to develop.

FIG. 11.—Transverse section of basidiospore of *G. applanatum*, showing same stage of endospore development as in fig. 9.

FIG. 12.—Transverse section of basidiospore of *G. applanatum*, showing same stage as in fig. 10.

FIG. 13.—Basidiospore of *G. applanatum* in longitudinal section, showing chitin reaction of the endospore; note swelling of both endospore and epispor, former giving a reddish violet color reaction.

FIG. 14.—Basidiospore of *G. applanatum* in longitudinal section, showing chitin reaction as in fig. 13; in this case hyaline beak has collapsed.

FIG. 15.—Basidiospore of *G. applanatum* in longitudinal section after having been heated in 20 per cent H_2SO_4 which has completely dissolved the epispor.

FIG. 16.—Basidiospore of *G. tornatum* in longitudinal section after being heated in 5 per cent H_2SO_4 , giving same result as shown in fig. 15.



1



2



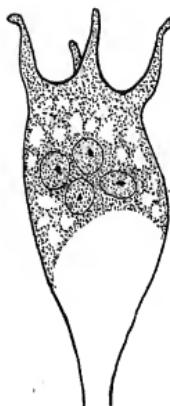
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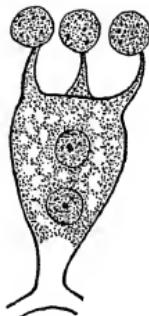
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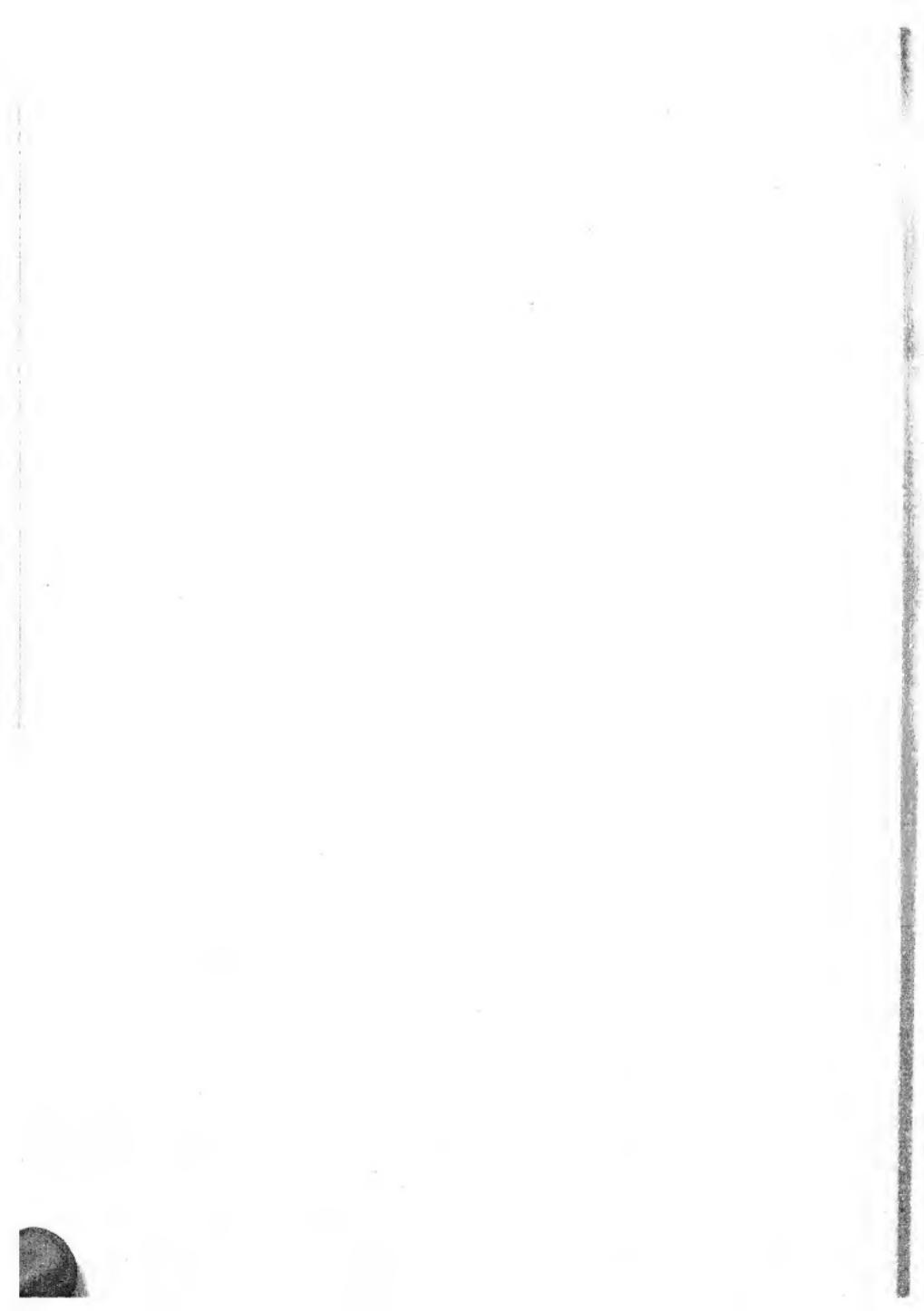
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CORRELATION OF COAL FLORAS IN HENRY COUNTY,
MISSOURI, AND THE NARRAGANSETT
BASIN¹

EDA M. ROUND

(WITH THREE FIGURES)

Of all the plant-bearing fossiliferous areas in North America, the region of Henry County, in Missouri, presents the most points of interest to students of the coal flora of Rhode Island. The Missouri series was doubtless a product, geographically, of the same type of marsh conditions which prevailed along the borders of the Great Interior Basin during the Carboniferous, when the Pennsylvania, West Virginia, and Illinois coal was formed; but the comparison of its stratigraphic sequence with that of Rhode Island shows that the two areas were evolved under entirely different conditions, in spite of which many similar plant species developed (table I).

Comparative conditions of deposition

Concerning the Missouri strata, WINSLOW (9) states:

The sandstones are of white, drab, yellow, and reddish colors with impressions of leaves and stems. . . . The limestones are sometimes in massive beds but always of a fine compact texture. . . . Beds of coal thin out and disappear; beds of shale pass into sandstone or grade into limestone.

WOODWORTH (7) states of the Rhode Island coal:

Alternations of fine and medium quartz, quartzite, and granite pebble conglomerates [occur] with pebbly sandstones, sandstone (graywacke), shales, and coal beds, becoming metamorphic southward. Colors: black, blue, green, gray, locally red.

The first point made by WINSLOW calls attention to reddish beds containing plant fossils. This differs from conditions in Rhode Island, where the Wamsutta series leads into the Coal Measures proper and contains actual red beds showing phytiferous impressions. The presence of limestone deposits, however, intercalated be-

¹ Work started in partial fulfillment for the degree of Ph.D. in the Department of Geology, Brown University.

TABLE I
SECTION IN HENRY COUNTY, MISSOURI AND IN NARRAGANSETT BASIN

HENRY COUNTY, MISSOURI				NARRAGANSETT BASIN	
Group	Formation	Stratum	Generalized section	Group	Local areas
	Pleasanton formation	Undifferentiated	Pawnee limestone Lambette shale Fort Scott limestone	Rhode Island Coal Measures	Westville shales Seeksink sandstone Aquidneck shales Ten-mile River beds Kingstown series Mansfield coal beds Cranston coal beds Sockanosset sandstone Pawtucket shales
Des Moines	Henrietta formation				Wamsutta slates and shale, red, gray Attleboro sandstones Wamsutta conglomerates
	Cherokee shales	Undifferentiated			

tween the carbonaceous shales, is not a feature of the Narragansett Basin (the Wamsutta series excepted), although the rule in Missouri. This difference may be explained by postulating a series of fresh water lakes, marshes, or even playas throughout Rhode Island during the Coal Age; while Missouri, likewise under the influence of fresh waters, was visited at intervals by marine invasions which left behind limestone beds and occasional marine fossils. WHITE (8) refers to the Missouri formations as follows:

The deposits of the lower portion of the Mesocarboniferous occurred during a period of terrestrial subsidence and advance of the shore line, the result of which is the theoretically complete concealment of earlier beds of the Coal Measures beneath the landward overlaps of succeeding sediments.

The lower members of the Rhode Island Coal Measures show expansion, restriction, or possible extinction of the swampy areas, along presumably fresh water lakes, where the Wamsutta beds overlap the Pondville arkose. The Coal Measures proper, however, although they appear to have developed under varying conditions, yet show, from the evidence of the plant species, a marked tendency to uniformity throughout the area. Table II shows the different fossil plants found to be common to Missouri, in Henry County, and the Narragansett Basin, with the localities from which the specimens were obtained.

Listed specimens

Considering each specimen in the order listed, a few observations concerning local conditions in the Narragansett Basin may be of interest.

CALAMARIALES.—The Calamariales are represented in Rhode Island by many large Calamites, among which are *Calamites cistii* Brgt. and *C. suckowi* Brgt. The leading deposits of these giant forms seem to be in the East Providence section of the state, where they now appear largely in the coarse sandstone layers into which they sank, presumably near their original places of growth, or to which they were carried by water currents of braided or distributary streams. Fruiting cones like *Calamostachys ovalis* Lx. are found from Pawtucket, only a few miles distant from East Providence. Various leaf forms like *Calamocladus longifolius* (Stb.) Brgt. and *C. equisetiformis* (Schl.?) Brgt. are very common, the latter being found in at

TABLE II

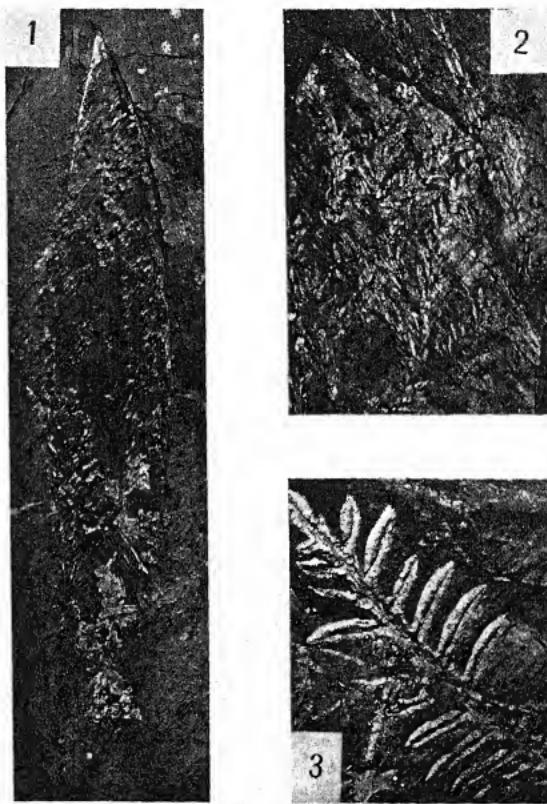
least sixteen of the fossiliferous sections of the Narragansett Basin. *Annulariae* are sparsely represented by forms resembling *Annularia ramosa* Weiss, whereas *A. sphenophylloides* (Zenk.) Gutb. and *A. stellata* (Schl.) Wood are common.

SPHENOPHYLLALES.—The Sphenophyllales common to Missouri and Rhode Island are *Sphenophyllum cuneifolium* (Stb.) Zeil. and *S. emarginatum* Brgt., both of which are fairly widespread, *S. (Calamocladus ?) fontinalis* nom. nov., rather local in habit, and *S. majus* Brunn., which is occasionally found. *S. (Calamocladus ?) fontinalis*, nom. nov. (fig. 2) appears in the Missouri flora as "*Sphenophyllum (Astrophyllites ?) fasciculatum* (Lx.)" (8), a preempted name and therefore changed to a new specific designation from its resemblance to the modern water moss *Fontinalis*.

LEPIDODENDRALES.—The Lepidodendrales are evidenced among Rhode Island fossils principally by their leaves, fruiting cones, and roots. *Lepidophyllum missouriense* White (fig. 1) was regarded by WHITE as typical of Missouri alone, and its appearance in Rhode Island broadens the geographical range of the species. *Lepidostrobus jenneyi* White and examples of Sigillarioid leaves are found from Pawtucket, while *Stigmaria sicoides* (Stb.) Brgt. has appeared from several localities around Providence.

FILICALES AND CYCADOFILICALES.—The Filicales and Cycadofili-
cales common to Rhode Island and Missouri are greater in number
and variety than any other plant group. They naturally divide into
several classes, the first being characterized by variously incised and
highly ornate forms as follows. *Eremopteris missouriensis* Lx. is occa-
sionally found in Rhode Island, although rather common in Missouri.
Pseudopeccopteris obtusiloba (Brkt.) Lx. is somewhat abundant from the Pawtucket Valley Falls section. *Sphenopteris Brittsii* Lx., as found in Pawtucket, contains bristly, irregularly incised pinnules from a broad secondary rachis, while the Missouri forms are more regular. *Sphenopteris capitata* White and *S. chaero-
phyloides* (Brkt.) Presl. appear somewhat alike as illustrated in the
literature of paleobotany; but specimens of the latter, as identified
by LESQUEREAUX, show fossils of a type found in abundance from
the Portsmouth section of the Narragansett Basin, while *S. capitata*
White is more sparsely represented in the state. *S. cristata* (Brkt.)

Presl. is mentioned in nearly all printed lists of Rhode Island fossil plants. Illustrations of this species from Europe, where it occurs



Figs. 1-3.—Fig. 1, *Lepidophyllum missouriense* White; location, Valley Falls, R.I. (no. 376, Brown University Collection); fig. 2, *Sphenophyllum (Calamocladus?) fontinalis* Round; location, Portsmouth, R.I. (no. 394, Brown University collection); fig. 3, *Alethopteris serlii* var. *missouriensis* White; location, Pawtucket, R.I. (no. 731, Brown University collection); photographs natural size.

sparingly in Middle and Upper Coal (3), show great diversity of shape and size. Specimens from Rhode Island may be found which resemble either the lax or close European forms, and may be re-

garded as having varietal differences only from those in other parts of the world. Fragments of *Sphenopteris illinoiensis* White are occasionally found from the Providence section of the state, but can hardly be regarded as common. *Aloiopteris erosa* (Gutb.) White and *A. winslowii* White are frequently seen among fossils of the Narragansett Basin. They are likewise characteristic species of Missouri.

In contrast to the highly dissected Sphenopterids and allied genera are the Pecopterids, which are characterized by simple, entire pinnules attached to the rachis by entire bases. The *Pecopteris* group is well represented in Rhode Island, among the most common of these being the one called by WHITE *Pecopteris arborescens* (Schl.) Brgt., which he hesitates to designate as a new species (8). It is inclined to be slightly larger than typical *P. arborescens*, and agrees in size with ZEILLER's *Pecopteris paleacea*. Specimens, however, show no more trace of the bristly rachis so characteristic of *P. paleacea* Zeil. than is described by the same writer for *P. arborescens* (Schl.) Brgt. (6). It appears to have been abundant in Lower Coal, Upper Coal, and Permian horizons of Europe (3). Another Pecopterid identified by LESQUEREAUX from certain Rhode Island material is *P. candolleana* Brgt. While occasionally seen, it is not found in as great quantities in the state as is the case with *P. arborescens* (Schl.) Brgt. Although this species is reported from the Upper Coal and Permian horizons of Europe, it is likewise not abundant (3). *P. dentata* Brgt., however, is very common in the fossil floras of both Rhode Island and Missouri; and its presence in Europe seems rather typical of Lower Coal, although it is found in Upper Coal and Permian (3). *P. (Asterotheca) hemitelioides* Brgt. is of frequent occurrence in the Narragansett Basin, especially around Newport. Its presence in Missouri is recorded with reservations (8). The form of *P. pseudovestita* White (5), characteristic of Rhode Island, appears much like *P. vestita* Lx. (5), except that hairs are not especially noticeable and the nervils are inclined to fork but twice.

The Alethopterids are fairly numerous in Rhode Island. While *Alethopteris ambigua* Lx. is occasionally seen, *A. serlii* (Brgt.) Goep. is rather common. *A. serlii* var. *missouriensis* White is found in the Pawtucket Valley Falls section of Rhode Island (fig. 3), and is an

interesting connecting link between the Narragansett Basin and Missouri floras, the variety having been thought typical of Missouri alone. WHITE states:

The varietal distinction of this Missouri form, which I have thought might be of stratigraphic utility, must be regarded as tentative, the question of its survival or elimination depending on the results of further study of material from other portions of the American Carboniferous.

Two Neuropterids are common to Rhode Island and Missouri. *Neuropteris rarineris* Bunn. has been taken from the Valley Falls mines in considerable quantities, iron having entered into the preservation of the plant in such a way that it appears as a brownish fossil on a background of black slate. *N. scheuchzeri* Hoff., the broad type, is found from Pawtucket, while the narrow form is common from Mansfield.

Occasional specimens of *Aphlebia* appear in the Narragansett Basin, among which *A. germari* Zeil. is a striking example. The specimens from Rhode Island are somewhat smaller than those figured from Missouri, however, although similar in shape.

CORDAITALES.—The only representatives of this class which have appeared thus far in the Narragansett Basin are the Cordaitales. Among these, *Cordaites communis* Lx., which WHITE finds difficult to distinguish from *C. borassifolius* (Stb.) Ung., is found to be fairly common in Rhode Island. The majority of specimens are imperfectly preserved, however, so that care is necessary to find the fine striae which characterize the species.

Summary of similar species

Taken as a whole, over 50 per cent of the Rhode Island fossil plant species are found also in Missouri. Since the Cherokee shales (from which the Missouri series was derived) are considered as belonging to Middle Pennsylvanian, probably Upper Westphalian in the nomenclature of Europe (4), there seems to be ample ground for concluding that the Rhode Island coal measures are akin to the Allegheny beds of Pennsylvania, the Cherokee shales of Missouri, and the Upper Westphalian of Europe.

139 SUPERIOR STREET
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EVAPORATION RATES IN A NORTH FLORIDA HAMMOCK

FRANK THOME

(WITH THREE FIGURES)

The term "hammock" is used in northern peninsular Florida to designate any tree community dominated by angiosperms, as distinguished from pinelands and cypress swamps. Common recognition takes account of two general types of hammock characterized both topographically and by their vegetation. A "low hammock" is a swamp forest, with *Nyssa biflora*, *Acer carolinianum*, and *Magnolia glauca*¹ dominant, their roots covered with standing water throughout most of the year. A "high hammock" is a ravine forest of mixed hardwoods, with species of *Quercus* and *Magnolia* dominant, on moist but well drained soil. These ravines are shallow, seldom more than 10-12 m. deep. Sinkholes with bottom drainage also support "high hammock" on their steep sides; in these a depth of as much as 30-40 m. may be reached. A third type of hammock is sometimes designated "oak hammock," consisting of evergreen oaks of the *Quercus virginiana* habit, on dry sandy soil.

A notable feature of the high hammocks is the relative scarcity of herbaceous and shrubby undergrowth. In the high hammocks the trees stand close together and their tops interlace in competition for light. Lianas abound, but other lesser plants find a place only in partial clearings and around the margins of the forest. Except for dead leaves the ground under the trees is bare, since apparently not enough sunlight falls through to nourish any smaller vegetation.

In both low and high hammocks part of the trees are evergreen and part are tropophytic, so that the canopy becomes somewhat thinner during the winter months, although of course it never approaches the degree of nakedness of a northern hardwood forest in winter and early spring. It was partly a desire to learn the influ-

¹ The nomenclature here followed is that of BRITTON, N. L., *North American trees*. 1908.

ence of this partial denudation on evaporation rates, and partly a curiosity as to the drought conditions that must be faced by broad leaved evergreens (for winter is the dry season in Florida), that led to the present study.

Terrain

The area selected is a small high hammock lying in the grounds of the Agricultural Experiment Station at the University of Florida, at Gainesville. The ravine runs almost due north and south; its bottom is about 8 m. below the level of the surrounding country. The soil is very loose and sandy, with a slight enrichment of humus.

The tree population is young and very mixed. *Quercus laurifolia* and *Magnolia grandiflora* predominate, but there are also considerable numbers of *Q. nigra*, *Q. virginiana*, *Hicoria alba*, *Celtis mississippiensis*, *Liquidambar Styraciflua*, *Magnolia glauca*, *Tilia pubescens*, etc. There are a few scattering pines, mostly *Pinus echinata* and *P. caribaea*. The larger trees stand densely on the eastern edge of the ravine, and extend out on the level ground for a short distance. In this upper part there are few trees of secondary size and no shrubs. Along the slopes of the ravine, however, the forest growth is open enough to permit an irregular second stratum of trees, consisting mostly of *Carpinus caroliniana*, *Crataegus* spp., *Morus rubra*, *Myrica cerifera*, and *Aralia spinosa*. A few shrubs also come in, such as *Callicarpa americana*, *Sambucus canadensis*, and *Sabal palmetto*. The chief lianas are *Smilax* spp., *Vitis cordifolia*, and *Rhus radicans*. A scattering display of ferns, a considerable growth of an escaped *Begonia* species near the brook, and on the drier slopes a small stand of *Galinsoga parviflora* from a weed filled fallow on the western edge of the hammock complete the picture.

Instruments and methods

To measure the evaporation conditions near the ground level, five radio-atmometric pairs of Livingston standard spherical atmometer cups were set up on an east-and-west transect of the ravine, the cups in each instance being about 20 cm. above the surface of the ground. Station 1 was located at the boundary between the hammock and the fallow field on the west. Station 2 was about 15 m. to the east of Station 1 and about 1 m. lower, in a thin stand of

elderberry bushes. Station 3 was placed in the most moist and shady spot that could be found, on springy ground, under a dense stand of elderberry about 1 m. above the level of the brook at the ravine bottom. Station 4 was erected in a partial clearing near the top of the eastern slope of the ravine, and Station 5 some 20 m. to the east, on the densely shaded, plantless floor of the groove.

A vertical series of radio-atmometric pairs of cups was also operated, to measure the evaporation conditions of the higher aerial levels. Station I was hung by means of a light rope over the limb of a giant pine tree above the general level of the foliage canopy of the grove; the height is estimated at about 18 m. Station II was placed in containers nailed to the trunk of a tree in the hammock, at a height of about 4 m. Station III was identical with Station 5 of the ground level series.

It was planned originally to operate the atmometers continuously from mid-October until the end of April, but a severe freeze coming unexpectedly during the first week in January wrecked the first set of cups, and continued cold weather during the remainder of that month made operation inadvisable. After operation was resumed, threats of cold waves made several shorter interruptions necessary during February. It is believed, however, that sufficient data have been accumulated to be of some value as an index to winter evaporation rates in this region.

It is much to be regretted that circumstances did not permit the securing of parallel data on the water-supplying power of the soil. Figures on evaporation rates alone are admittedly incomplete, giving only one-half of the story, but this defect may be somewhat mitigated in the present instance by the fact that casual observation showed that the soil in all parts of the area studied always carried available water close to the surface.

Discussion

The first thing noticeable about the graphs is their lack of any definite indication of a seasonal march of evaporation rates. General evaporation rates were higher in November than in October; higher in March than in April. Some such result as this of course was only to be expected in a region of equable winter climate. Could the unfortunate hiatuses due to the abnormally cold midwinter weather

have been avoided, it is doubtful whether the data for this period would have been materially different. The periods of low evaporation, notably the one in December, correspond generally with spells

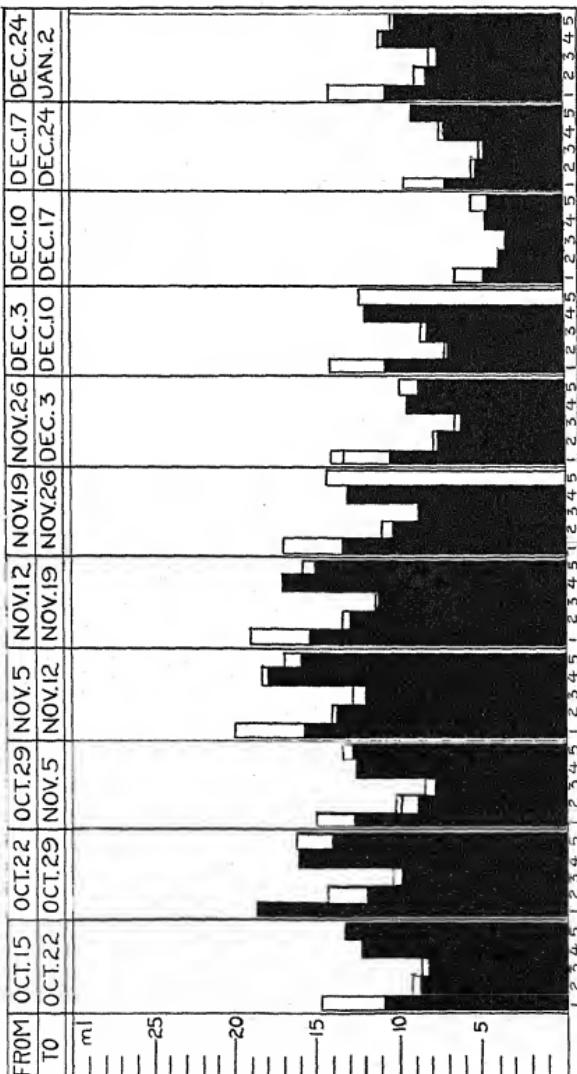


FIG. 1.—Mean daily rates of evaporation in millimeters from standard spherical black and white atmometers in ground level transect series: black columns indicate simple atmometric effect; white extensions indicate increased evaporation due to direct insolation; absence of white extension indicates identity in reading of black and white instruments; in a few instances the reading of the black was less than that of the white.

of cool, foggy weather. The general evaporation rates seem to be high enough to indicate that winter, a season of scanty rainfall in Florida, must be a real drought period, for which the deciduous habit

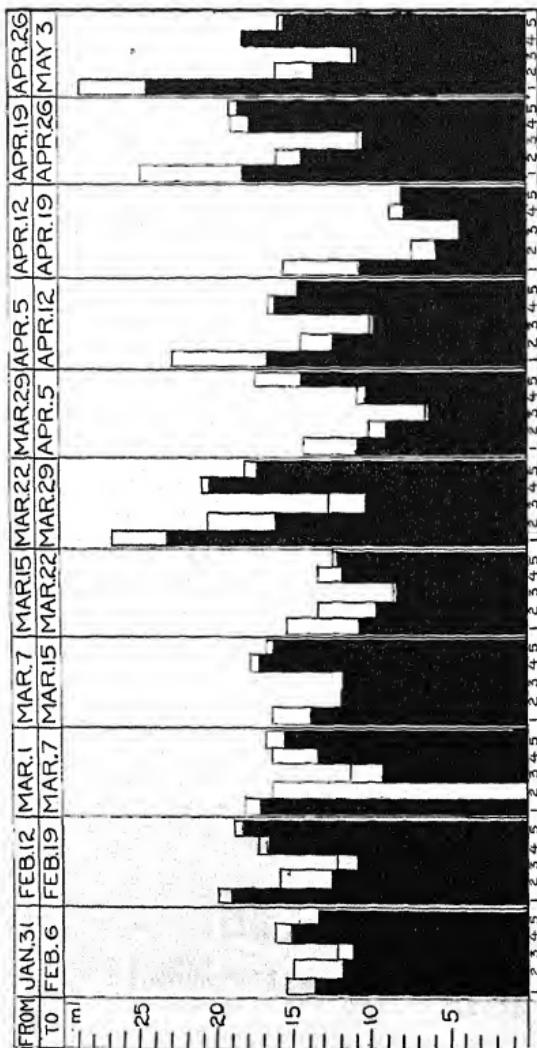


FIG. 2.—Continuation of fig. 1

of some of the trees, and the semixerophytic structures of others, are apparent adaptations.

Results ascribable to influences of topography are entirely ortho-

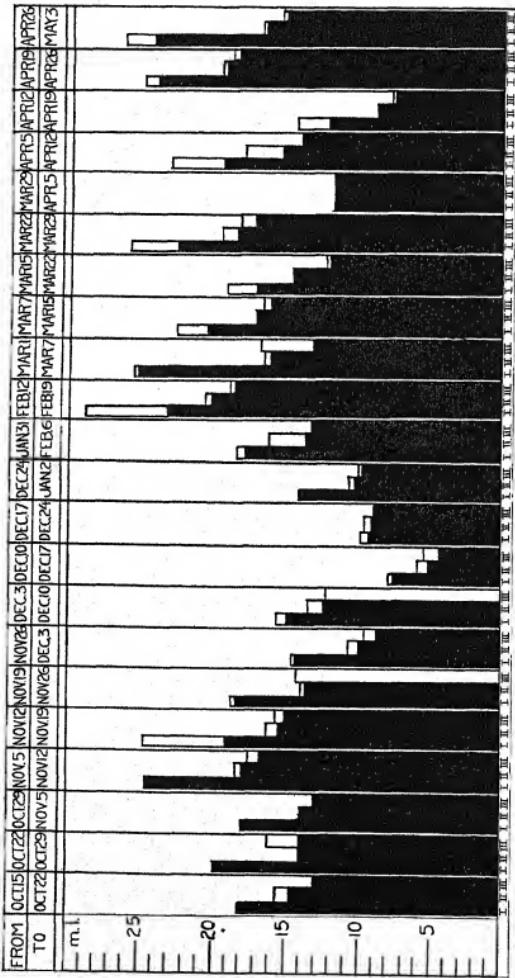


FIG. 3.—Mean daily rates of evaporation in milliliters from standard spherical black and white atmometers in vertical "aerial" series; conventions as in fig. I.

dox. In figs. 1 and 2, each set of weekly curves might almost constitute a fair profile of the transect along which the stations were arranged, with Station 3 at the bottom of the ravine.

The really interesting feature about the data here presented is the very slight radio-atmometric effect observed at stations under the trees, even during the period of maximum denudation. The only station where the readings of white and black bulbs differed at all significantly was Station 1, situated in the open fallow field, where the added evaporation due to direct insolation amounted in most instances to between 20 and 30 per cent. At all stations sheltered by the trees the differences were sporadic and mostly insignificant; in a few instances there was even a slight excess of evaporation from the white cups. When this situation in partially evergreen woods is contrasted with that found by the writer in a deciduous forest in central Illinois,¹ where there was a high radio-atmometric effect in early spring that disappeared as soon as the leaves developed, the importance of the foliar canopy in reducing the influence of sunlight on evaporation rates on the forest floor becomes strongly apparent.

The results from the vertical series of stations (fig. 3) are also fairly normal, the evaporation rates bearing a direct relation to the distance above the ground. The excess of the evaporation rates in the air above the general treetop level over that beneath the foliar canopy was perhaps not as great as might have been expected, but it was always quite definite. The relatively insignificant radio-atmometric effect at Station I, high above the treetops, is somewhat anomalous, especially when compared with that at the ground Station 1, in the fallow field. This is probably accounted for by the fact that it was impossible to hang this station so that it could get the full benefit of the afternoon sun.

The writer is indebted to the Department of Biology of the University of Florida for facilities used in pursuing this study, and to the Department of Botany of the University of Chicago for the loan of apparatus.

SCIENCE SERVICE
WASHINGTON, D.C.

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¹ THOME, FRANK, Ecological factors in region of Starved Rock, Illinois. *BOT. GAZ.* 74:345-368. 1922.

SIGNIFICANCE OF TRACES OF ELEMENTS NOT ORDINARILY ADDED TO CULTURE SOLUTIONS, FOR GROWTH OF YOUNG ORANGE TREES¹

A. R. C. HAAS AND H. S. REED

(WITH SIX FIGURES)

The purpose of this paper is to call attention to the fact that long continued use of certain nutrient solutions, commonly considered complete, may produce injurious effects which can be removed by the addition of traces of elements ordinarily considered unessential for plant growth. Students of plant nutrition who have employed cereals may have failed to encounter this difficulty, because the seeds, or the inevitable impurities of the chemicals, or the solubility of the containers have supplied the quantities of these elements necessary for short periods. Investigators in this field of experimentation have frequently ascribed the deleterious action of solutions to the toxicity of the distilled water employed. As will subsequently be shown, the deleterious action of the nutrient solutions used in this experiment was not due to traces of the heavy metals, or to volatile organic compounds, because all water was carefully treated with carbon-black, a powerful absorbing agent.

In the course of experiments on the effects of salts upon the growth and composition of orange trees, it was found that a characteristic type of injury usually appeared after 18-24 months. There is no evidence that the bad results were due to an accumulation of materials in the sand cultures, because the containers were well drained and the cultures were brought to their optimum moisture content with distilled water before the first addition of the nutrient solution. As each later supply of nutrient solution percolated downward through the sand, it displaced practically an equal volume of the old solution. Moreover, the harmful effects on the trees were overcome, not by the use of materials of greater purity, but by the addition of something previously omitted.

¹ Paper no. 145, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

It was found that every orange tree (several hundred in all) in sand cultures in asphalted galvanized iron cans that received culture solutions made with distilled water showed symptoms of decline, while orange trees in large tank cultures of sand receiving a culture



FIG. 1.—Orange tree showing initial stages of injury: tree grew in sand culture which received "complete" nutrient solution; reflexed leaves and leafless shoots on right of picture; following addition of "*A-Z*" to nutrient solution, all affected leaves shed and normal growth resumed.

solution made with tap water showed no decline. The injurious symptoms were produced alike, whether the nutrient salts were dissolved in ordinary distilled water or in distilled water treated with "Elf" brand carbon-black.

Fig. 1 shows the beginning stages of decline in a young Valencia

orange tree which had received Hoagland's solution in sand culture before remedial measures were taken. A few of the shoots had been defoliated, and such shoots usually died back to the end of the preceding growth cycle. Trees which received unfavorable salt addi-



FIG. 2.—Young orange tree showing advanced case of injury: tree grew in sand culture two years and received culture solution containing 500 p.p.m. sodium as sodium sulphate; later the leaves here shown were shed, but normal new growth was produced by adding "A-Z" to nutrient solution.

tions in their culture solutions developed the decline more rapidly than those which received Hoagland's solution. Fig. 2 shows the more advanced injury of a Valencia orange tree which grew in a sand culture two years with a culture solution containing 500 p.p.m. sodium as sodium sulphate. The injured leaves curled downward

along the midrib (fig. 3). The leaves were pale yellowish green on the upper side, and were marked with numerous spots which on the lower side seemed to be oil glands having a faded appearance. Frequently the center of such spots took on a resinous appearance (fig. 4).

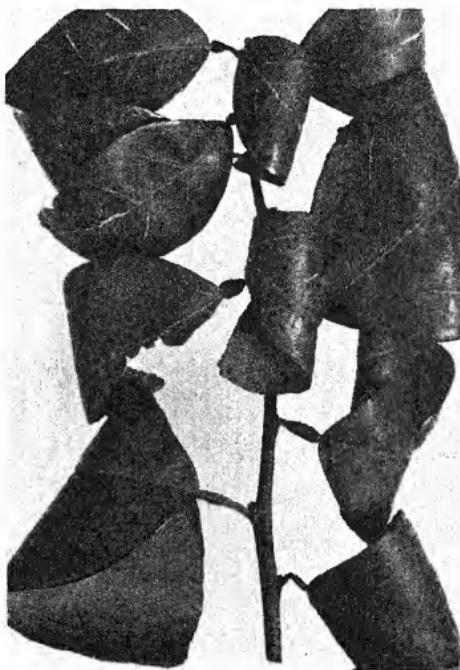


FIG. 3.—Orange leaves which were recurved and show the thick veins, many of which had split open.

In addition to the curling and spotting of the leaves, many of them became corky and split along the veins. Fig. 5 illustrates the extent to which such vein injury may proceed. The veins seem to become riblike and extend well above the surface of the leaf, giving the intervenous region a shrunken or dried appearance. Seedlings of Florida sour orange grown in crocks of sand in the glasshouse also

showed such effects after four or five years. Leaves are shed prematurely once the veins have become split and corky.

When the decline became severe many of the leaves were shed, followed by a new growth, although the new leaves usually fell prematurely. The trees were grown in large containers and were not root bound. The sand was kept at optimum moisture content.

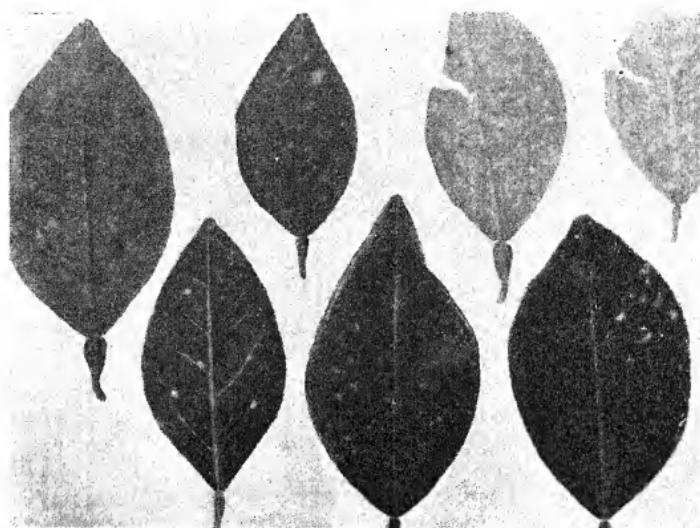


FIG. 4.—Orange leaves containing yellowish spots whose centers often had resinous exudations.

As the injury progressed, and after many of the younger cycles of growth had died, groups or clusters of buds (multiple buds) were produced in the leaf axils or above the former leaf scars, but none of them seemed able to develop (fig. 6). It was observed that the trunks of most of the trees which received large amounts of calcium chloride or calcium nitrate in their culture solutions in sand cultures showed considerable gum exudation, but this symptom was absent in later cultures where the deficiencies in the solutions had been supplied.

Since the nutrient solutions made up with tap water produced

healthy growth, the first thought was that the tap water contained something that the distilled water lacked. Furthermore, the trees used were budded stock, and when placed in the sand cultures were pruned back to a short piece of trunk and root, and the symptoms made their first appearance during the second year's growth when presumably the supply of constituents originally present in the trunk or root was depleted.

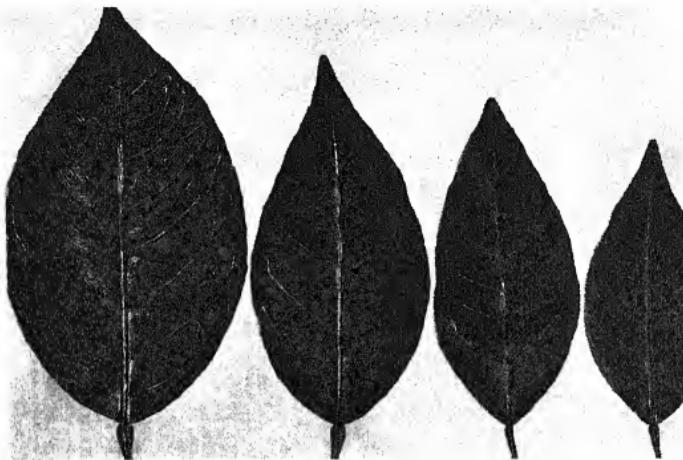


FIG. 5.—Orange leaves whose midribs and veins were greatly thickened and in many cases split open.

Since many of the older trees were beginning to show symptoms of a possible deficiency, the chief concern at the time was to remedy this situation, and then later to ascertain just what constituted the deficiency. A suspension, called for convenience "A-Z," was added to the culture solutions or to the distilled water applied to the sand cultures, so as to give a concentration of 0.2 p.p.m. of Al, I, Ti, Br, Sr, Li, Mn, B, and NH₄ respectively. The salts employed were aluminum sulphate, potassium iodide, titanium sulphate, potassium bromide, strontium nitrate, lithium nitrate, manganese sulphate, boric acid, and ammonium nitrate. Within a week no further curling of leaves occurred, although all badly curled leaves eventually

were shed. New growth was soon in evidence, and in a few weeks none of the symptoms of deficiency remained.

These results harmonize with those of other investigators (1, 2, 3, 4, 5, 6) who have found that certain of the preceding elements are actually required for the successful growth of plants.

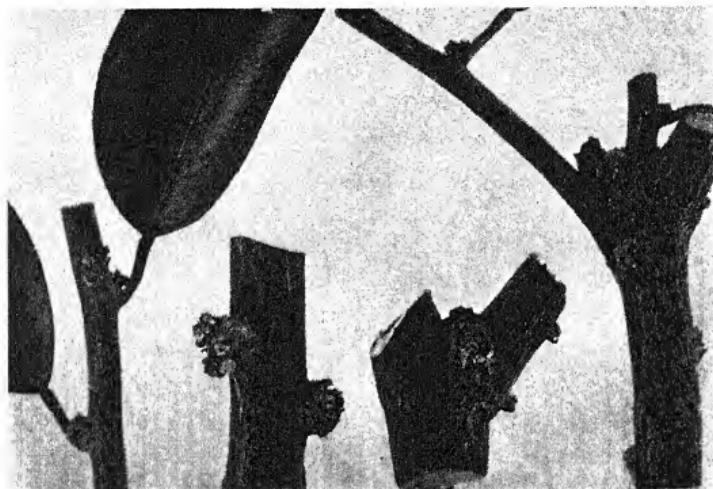


FIG. 6.—Pieces of orange shoots from injured trees on which there had been a formation of multiple buds.

At present it has not been discovered which of these ions was responsible for the prompt improvement of the trees, nor do we know that all of the symptoms were due to the deficiency of only one ion. A more detailed investigation of the problem is under way.

CITRUS EXPERIMENT STATION
RIVERSIDE, CAL.

[Accepted for publication June 3, 1926]

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CHROMOSOME NUMBERS IN BUCKWHEAT SPECIES¹

KARL S. QUISENBERRY

(WITH PLATE VI AND SEVEN FIGURES)

Very little cytological or genetical work on buckwheat has been reported in this country. This is probably due to the fact that the crop is one of minor importance, usually being grown on the poorer soil, since it does not compete with the important cereals on the better soils. Because of the possibility of starting a rather extensive inheritance and improvement project on this crop, it was felt desirable to learn something of the chromosome numbers in the various species, in order to obtain some indication of what might be encountered in the way of sterility of interspecific crosses.

It has been shown by various workers that the chromosome numbers of the several species of wheat, oats, and barley vary in multiples of a given number. The eight species of wheat, for example, fall into three groups with haploid chromosome numbers of 7, 14, and 21 respectively. In interspecific crosses considerable sterility is encountered, due in part to the differences in chromosome numbers. As a rule the greater the difference in chromosome numbers the greater the degree of sterility.

Materials and methods

According to LEIGHTY,² there are two, and possibly three species of *Fagopyrum*. The varieties Japanese, Silverhull, and Gray belong to the species *F. esculentum*. The first two varieties are the more important from a production standpoint, in this country. In some of the more or less mountainous regions Tartary buckwheat, *F. tartarium*, is grown under various varietal names. In general, the seeds of Tartary buckwheat are much smaller and less angular than those of Japanese or Silverhull. It is also interesting to note that several, if not all of the varieties of Tartary buckwheat are highly self-

¹ Scientific Paper no. 23, West Virginia Agricultural Experiment Station, Morgantown, W. Va. Approved by H. G. KNIGHT, Director.

² LEIGHTY, C. E., Buckwheat. U.S. Dept. Agric. Farmers' Bull. 1062. 1919.

fertile, setting seed abundantly under glassine bags. Varieties of *F. esculentum*, on the other hand, are quite highly self-sterile. The third species is *F. emarginatum*, which, according to LEIGHTY, is not known to be grown pure in this country. In this type the angles of the seeds are extended and form wide margins or wings. Some workers feel that this type or species should be considered as a variety of *F. esculentum*.

Studies were made on Japanese and Silverhull belonging to *F. esculentum* (C. I. 91), Notch Seeded belonging to *F. tartaricum*, and on one sample of *F. emarginatum*. Root tip material was taken from seeds which had been germinating between blotters for 36-48 hours. In the case of the Japanese variety, some plants were grown in the greenhouse in order to obtain pollen mother cells from which to make chromosome counts in the haploid condition.

The material was killed with Allen's modification of Bouin's fluid, commonly known as B-15, and run through the regular paraffin method. In order to obtain anthers which were in the proper stage to give pollen mother cells, all buds killed were first examined to determine their stage of development. The method was to remove some anthers from buds at the base, center, and tip of the raceme branch, and mount these by Belling's method. As a rule the anthers from the tip of the raceme branch showed stages too young, while the base might show anthers containing pollen grains, since the inflorescence of buckwheat is indeterminate in habit of growth. The entire raceme branch was killed and imbedded, assuming that some buds would be in the right stage. Technique was not developed whereby chromosome counts could be made by Belling's method, due in part to the fact that the chromosomes are very small. Root tip sections were cut $5\ \mu$ thick, while the sections of pollen mother cells were cut $7.5\ \mu$. Haidenhain's iron-alum haematoxylin stain was used.

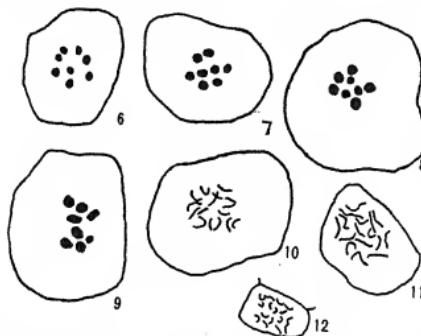
The photomicrographs were taken under a Bausch and Lomb 2 mm. immersion objective having a numerical aperture of 1.25. A daylight filter was also used to aid in obtaining definition.

Results

In the varieties and species studied no differences in chromosome numbers were found. In all cases the diploid chromosome number

obtained was sixteen. In the pollen mother cells from Japanese, counts were obtained showing a haploid number of eight. The counts obtained on Silverhull and Japanese agree with those reported by STEVENS.¹ Figs. 1–5 show photomicrographs of some of the material, figs. 1 and 2 being sections from pollen mother cells of Japanese. Fig. 3 shows the diploid chromosome number for Japanese, while figs. 4 and 5 are from *F. emarginatum*.

STEVENS reports that in the anaphase of the heterotypic division the eight chromosomes of a short styled plant tend to be arranged



FIGS. 6–12.—Figs. 6, 7, pollen mother cells from same anther of Japanese; figs. 8, 9, pollen mother cells from short style plant of Japanese; fig. 10, cell from root tip of Notch Seeded *F. tartaricum*; fig. 11, cell from root tip of Japanese; fig. 12, cell from root tip of *F. tartaricum* (C. I. 91); $\times 2300$.

with six in the peripheral ring and two in the middle; while in the long style form the arrangement is seven in the peripheral ring and one in the middle. From the results of the investigation here reported no such conclusion could be drawn. In fig. 6 the arrangement may be considered as having seven in the peripheral ring with one in the middle; while in fig. 7 it may be considered that there are six in the peripheral ring and two in the middle. Both cells are from the same anther of a plant of unknown form of style. Figs. 8 and 9 are drawings from cells of different short style plants of Japanese. It would appear from these results that no definite arrangement of the

¹ STEVENS, N. E., Observations on heterostylous plants. BOT. GAZ. 53: 277–308. 1912.

chromosomes occurs in the heterotypic metaphase of the varieties studied.

No consistent variations in size of chromosomes were found within a given cell. In the drawings the differences in size are due to the fact that only parts of the chromosomes are included in the section.

The writer takes this opportunity to acknowledge the valuable advice and criticism offered by Dr. FRED GRIFFEE of the University of Minnesota, under whose direction the work was done.

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MORGANTOWN, W. VA.

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EXPLANATION OF PLATE VI

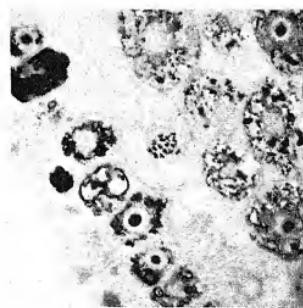
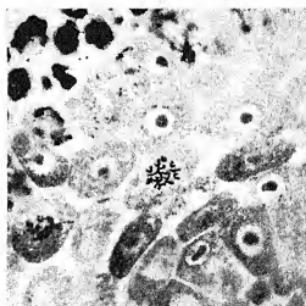
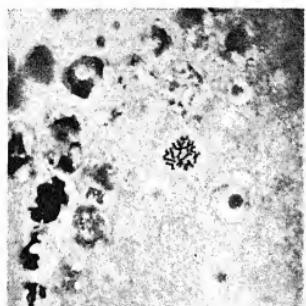
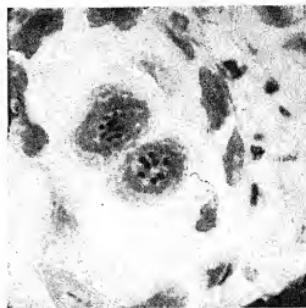
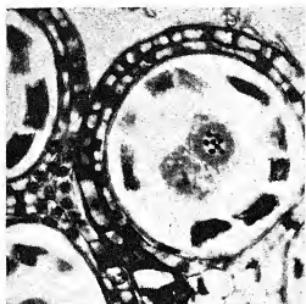
FIG. 1.—Pollen mother cell from Japanese, showing haploid number of 8.

FIG. 2.—Pollen mother cell from short style Japanese plant, showing haploid number of 8.

FIG. 3.—Chromosomes in cell from root tip of Japanese, showing diploid number of 16.

FIG. 4.—Chromosomes in cell from root tip of *F. emarginatum*, showing diploid number of 16.

FIG. 5.—Chromosomes in cell from root tip of *F. emarginatum*, showing chromosomes in late metaphase.



QUISENBERY on CHROMOSOME NUMBERS



DUNE FORMATION BY PINE BARREN PLANTS

ARTHUR PIERSON KELLEY

(WITH TWO FIGURES)

Topography

Northern New Jersey is a region of glaciated hills, while the southern part of the state is a region of undulating sands. The northern half is clothed with deciduous forest, while the major part of the southern half is so copiously set with pines that it has long been called the Pine Barrens. The forest floor of the Barrens is not level, but is ridged and hollowed in an apparently schemeless way. The topography is much like that of the gray dunes beside the sea, old dunes which have become fixed and stable and overgrown with matted *Hudsonia* and tangled thickets of oak and catbrier.

There are hills in the Pine Barrens, as around Whitings, where an elevation of 300 feet is reached, but these have had a rather different history, being apparently the remnants of land of greater age (8). The dune hills along the coast are the result of wind and wave action, as described by such writers as GERHARDT (5) for Germany, COWLES (3) for the United States, and COCKAYNE (2) for New Zealand. These dunes take the form of long ridges or chains extending parallel to the coast from which the sand is derived. There are areas, however, upon which are found hummocky hills scattered in endless profusion, especially in places which have been subject to dune action for a long time. Concerning such hills COCKAYNE (1) states:

Sandhills not forming chains may be either portions of such separated by wind action, or they may have originated directly on a sand-plain, or elsewhere, after the primary hills were destroyed or had wandered on. Sand-binding plants are chiefly responsible for the origin of these secondary hills.

Vegetation

Sand-binding action of plants seems to be responsible for the development of the Pine Barren hillocks. The plants of the Barrens have received much attention, and only the broadest ecological

groupings need be recalled here. *Pinus rigida*^x is the facies of the Barrens, but there are other trees present, such as *P. echinata*, *P. virginiana*, *Quercus stellata*, and *Q. marilandica*. *Q. ilicifolia* and *Q. prinoides* form bushy undergrowths on open areas among the pines, with ericaceous shrubs, as *Neopieris mariana* and *Gaylussacia baccata*. The ever present *Pteridium aquilinum* and *Baptisia tinctoria* cover wide areas, while an acid-loving consociation of perennial herbs is scattered to copious in the pine needle litter under the trees: *Fissipes acaulis* (in Middlesex County), *Chimaphila maculata*, *Pyrola rotundifolia*. In open spaces *Arenaria caroliniana*, *Tithymalopsis Ipecacuanhae*, and *Pyxidanthera barbulata* spread upon the sand, while *Hudsonia ericoides* (in Middlesex County *H. tomentosa*) forms broadly spreading mats.

Formation of scattered hills

The forest floor and the open spaces in the forest, then, are rather well covered with a carpet of vegetation or of vegetable remains. Even under the needle litter of the pines the sand is kept moist and secure from the wind. When a plant dies the sand beneath is exposed, for but little leafage has accumulated beneath the impoverished branches. The winds pick up the loose sand, and, when the breeze blows fresh, a trough is quickly scooped into the surface and the plants are undermined. This sand is then borne onward, scouring pine branches and trimming off fascicles which oppose its progress. It is finally deposited against some obtruding object and heaped into a small pile.

Trees of the Pine Barrens produce basal whorls of branches (fig. 1). This fact, while remarked by STONE (11) in *Pinus rigida*, does not seem to have been emphasized by any writer on the Barrens. It would appear to be a response to the greater intensity of light reflected from the sand, and a Pine Barren tree, even as a sapling, may be excurrent at the base and deliquescent at the top. The pines especially produce bushy growths upon the sand, which at first sight seem to be seedlings, but closer inspection shows that they are only upgrowing tips of branches. Such branches have not been found

^x All specific names are in accordance with Britton's *Illustrated flora*, 2d. ed.

rooting although covered with sand, but rooting of coniferous branches so placed has been mentioned (6).

Very gradually the basal branches are covered with slowly drifting sand, the ends continuing growth upward, as MACDOUGAL (7) noted in the Colorado desert, and COCKAYNE (2) in New Zealand dunes. Thus a basin is developed with the trunk in the center. A



FIG. 1.—*Pinus rigida* with basal branches; ground covered with snow

stray juniper "berry" or an acorn, as well as pine seeds drop in among the thickened branch ends, and seedlings thus develop, aiding in building the cone. Finally the original tree dies and the saplings continue its enlargement, with a resultant hill upon which frequently stand junipers and oaks. These hillocks are the antithesis of those noted by COWLES (3), which were the result of wind erosion, although some of the Pine Barren hills are the result of such erosion.

Formation of dunes

Not only hillocks are formed but long ridges which are really dunes, where pine forest meets deciduous forest. The Pine Barrens

extend northward into Monmouth County, while isolated patches exist in Middlesex County, one tiny area being on the terminal moraine of the last glacial advance. These isolated areas possibly represent the outer limits of an older Pine Barren area which has been and is being invaded by oak forest, which in itself is subclimax to beech forest. In one area near Spotswood, the two forests are so



FIG. 2.—Long dune where pine barren meets oak forest; ground covered with snow

arranged that the prevailing west wind sweeps first through the pine forest with its scattered trees, and then into the more copious growth of the oak forest. At the juncture a dune of from a few to twenty feet high has developed, as shown in fig. 2.

Earlier records of coniferous dunes

Coniferous dunes have been recorded from a number of places. On Cape Cod, plant succession on dunes was traced by WESTGATE (12), although he did not investigate the effect of plants on their development; *Pinus rigida* was noted as a subclimax stage to oak-beech forest. A coniferous dune was found by HARVEY on Cape Bre-

ton Island, while STOMPS (10) noted that coniferous forest usually develops on windward slopes and summits of Michigan dunes. In the same region COWLES (4) found coniferous and oak dunes covered with *Pinus banksiana* and *Quercus tinctoria* respectively. Miss SNOW (9) speaks of Delaware pine dunes as being antecedent to the present active dunes, "for the active crest shows knobs still held by pines, and the dead trunks falling on the windward side."

The Pine Barren hills seem to be what COWLES (3) calls "dunes of slow growth." These are "formed in older sands by less furious winds and different vegetation." *Juniperus sabina procumbens* was found to be a dune former within moderate limits, but pines were unable to withstand the advance of active dunes. In the New Jersey Barrens, however, dune action is so slow that the pines are able to keep pace with the sand deposition.

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INFLUENCE OF SALT UPON GROWTH RATE OF ASPARAGUS¹

WILLEM RUDOLFS

(WITH TWO FIGURES)

Introduction

In another paper² the results of a study of beans grown under definite conditions as to temperature and humidity showed a decided advantage when the plants were grown from heavier seeds than when they were grown from lighter seeds. The effect of a stimulant upon the growth curve seemed of decided interest.

In the course of an investigation regarding the effects of common rock salt on plant growth, experiments were made with asparagus. ROBERTSON's³ formula, considering growth as an autocatalytic chemical reaction, was fitted to the observed data in the construction of growth curves:

$$\log \cdot \frac{x}{a-x} = K (t-t_1) .$$

In this equation a is the final size of the plants; x is the size of the plants at time t ; and t_1 is the time at which the plants have reached half their final size, or when $x=a/2$; and k is a constant. It was thought that a comparison of the curves for plants grown under the influence of different amounts of salt would perhaps throw light upon the question as to that part of the growth cycle upon which salt (and possibly other fertilizers) exerts its greatest influence.

Experimental results

The experiments with asparagus were conducted on the horticultural farm at the New Jersey Agricultural Experiment Stations.

¹ Paper no. 317 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

² RUDOLFS, W., Influence of temperature and initial weight of seeds upon the growth rate of *Phaseolus vulgaris* seedlings. Jour. Agric. Res. 26: 537-539. 1923.

³ ROBERTSON, T. B., Further remarks on the normal growth of an individual, and its biochemical significance. Arch. Entwickl. Mech. 26: 108-118. 1908.

Plots were laid out in the middle of a field on which asparagus had been growing for two years. These fields, and subsequently the experimental plots, consisted of an equal number of rows running east and west. The area is level, and the soil is a Sassafras loam. The duplicate plots consisted of blocks comprising an average of eighty plants. In addition to approximately ten tons of stable manure and one ton of poultry manure per acre, applications were made of 150, 300, and 500 pounds of common rock salt respectively, and again in the following year these amounts of salt were applied in addition to ten tons of stable manure and one ton of poultry manure per acre. The first year two cuttings were made. Seven days after the last cutting, counts of the numbers of plants and stems per plant, and measurements of the latter were begun and continued throughout the entire season at intervals of 7-10 days. During the second half of the growing season the asparagus beetle did some damage on all plots, resulting in dead tips of the stalks. Late in the season accurate measuring became extremely difficult on account of the entangling of the branches, and measurements were finally stopped, but counting of stems was continued at intervals until the end of the season.

Fig. 1 shows the fitted curves for the length of the stems. By the "check" curve, observed data are represented by small circles. The fitting of the other curves was about the same as in the case of the check. The stems were measured separately, but for the calculation of these curves these separate readings were added together and divided by the number of stems per plant, and each reading in the data, from which the curves are calculated, represents the average length of about eighty plants. The regular increase in the total length of stems per plant reached a maximum in the middle of the season. A second growth period commenced, following essentially the same curve as is indicated in the first part of the curves.

The curves for the number of stems produced are given in fig. 2. Cycles similar to those in the curves constructed from measurements of length are obtained. The increase in numbers of stems was as regular and consistent as the increase of total length of stems. In comparing the curves for the total length of stems with the curves for the total numbers of stalks, it is evident that the difference in total length is mainly produced by an increase in the number of

stems per plant. When the greatest possible length of the growing stems is reached in the middle of the growing season, the plants will produce new shoots giving rise to new stalks. An increase of stalks

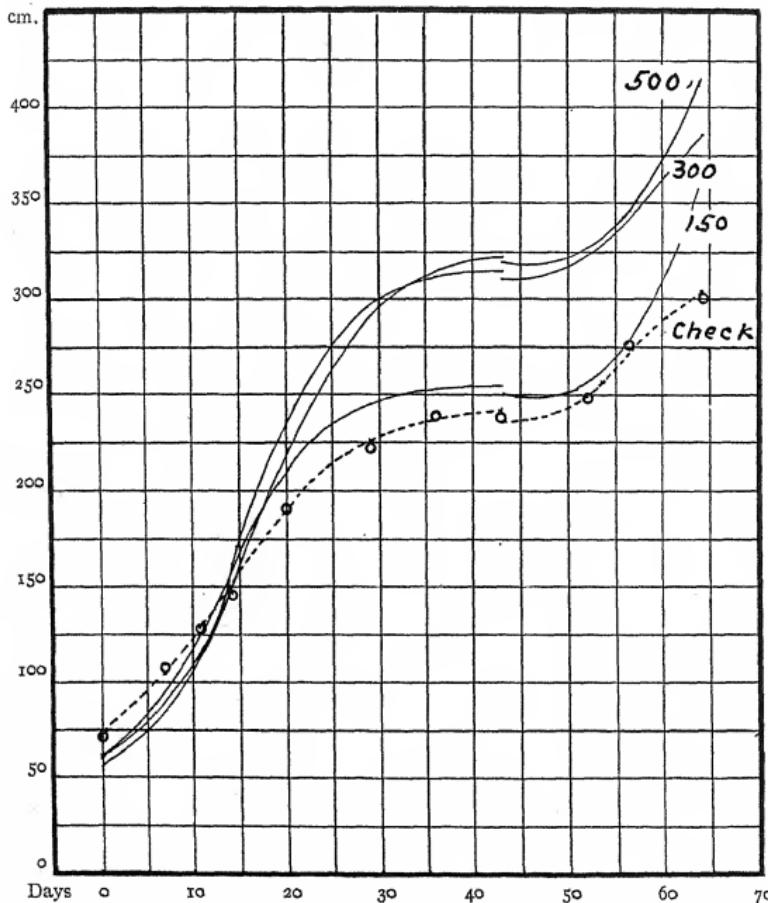


FIG. 1.—Growth rate of asparagus as represented by summated lengths of stems: plants receiving respectively none (checks), 150, 300, and 500 pounds of salt per acre. Curves show values obtained from averages of 80 plants. Apparent break of curve is due to necessity of fitting two successive growth cycles with different formulas. Data given in check curve illustrate closeness of fitting.

leads to an increase of storage food, and consequently to higher yields in the next season.

The influence of salt manifested itself mainly after the fourteenth day of measuring, that is, after three weeks of continuous growing.

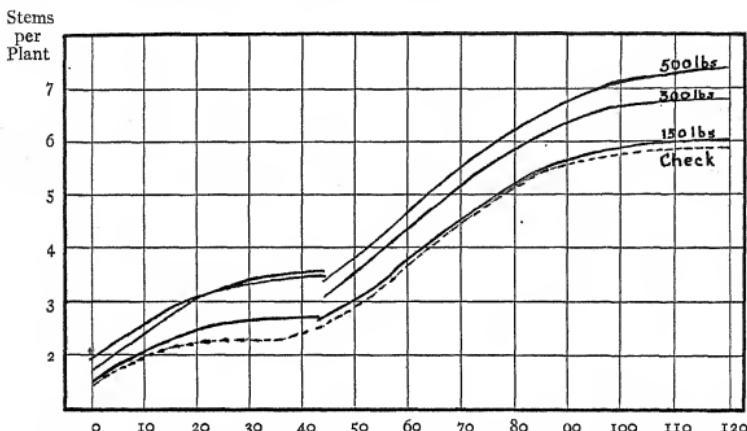


FIG. 2.—Growth rate of asparagus represented by numbers of stems per plant: curves show values obtained from averages of 80 plants.

This influence continued throughout the first cycle, and seemed to accelerate growth again in the course of the second cycle. From these curves it appears that salt is not merely a stimulant of growth at the beginning of the season, but continues to stimulate throughout the growing period of asparagus plants.

Summary

1. Experiments with common rock salt in addition to manure applications were conducted with asparagus in the field. Applications of 150, 300, and 500 pounds of salt were made for two years in succession. Measurements of the length of asparagus stems were taken, and the numbers of stems were counted at intervals of 7-10 days throughout the second season.

2. The asparagus showed two distinct cycles of growth in the season, and each cycle proceeded at a rate corresponding to an autocatalytic chemical reaction, producing an S-shaped curve.
3. Salt exerted a regular and comparable influence throughout the growing season upon the total length; and the number of stems of asparagus plants.

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A STUDY OF A MEXICAN RICCIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 363

M. ARLOUINE CHESEBROUGH

(WITH SIX FIGURES)

In October 1908, Dr. W. J. G. LAND found a species of *Riccia* growing at an altitude of about 2042 m., under a Nopal cactus on an exceedingly arid mountain side near Guanajuato, Mexico, a region the streams of which are so heavily charged with mineral salts that they are noticeably lacking in algae. These surroundings are so unique for this commonly hydrophytic or hydro-mesophytic genus as immediately to stimulate a desire to determine whether these plants showed any structural peculiarities, and, if so, whether these were of such a nature as to indicate any special fitness for growth in a particularly arid situation. Of this material, killed in the field and imbedded and stained in the ordinary way, LAND had already made a series of fifty slides. These he kindly lent to me for the present study.

The preparations available strongly suggest that the asymmetrical thallus is built from a single meristem initial, as shown in fig. 1. It lies well toward the ventral side of the thallus and cuts off segments, dorsal and ventral, sinistral and dextral. From the subsequent divisions of the dorsal segments is built the thick dorsal portion of the thallus. From the divisions of the ventral segments results the relatively small development of thin walled cells forming the ventral comparatively non-chlorophyllose portion of the plant. From the divisions of the sinistral and dextral segments the large wings are built. The cells in the region of the median line become rather elongate, and form a somewhat specialized region of food conduction.

The thicker dorsal part of the thallus is composed of exceedingly compact tissue arranged with great regularity, in the form of plates of cells separated by air chambers, deep clefts reaching to within one cell layer of the median line. These air spaces arise by internal

cleavage, as do the air chambers of *Riccia fluitans* and *R. natans* reported by Barnes and Land.¹ Splitting normally occurs early in the development of the meristematic region, about three rows of cells from the margin, between the superficial cells and those immediately underlying them, and the split may soon break out to the surface, as shown in fig. 2. The position of the first splitting, and the fact that the clefts never extend to the median plane which lies between the ultimate products of the dorsal and ventral segments of the wedge-shaped marginal cells, show that the splitting progresses from below upward, and not as Leitgeb² states or as Miss Hirsch³ reports for *Riccia Frostii*, from above downward. This is shown in fig. 1. The plates of chlorophyllose tissue resulting from further division and growth of the dorsal segments, and separated by such simple clefts, constitute a thallus indicative of a rather primitive condition of thallus architecture, for food must pass down each row of cells almost to the median line and thence to the points of consumption, instead of passing by a more direct route. Likewise this type of incomplete splitting, resulting in plates of cells rather than in filaments of chlorophyllose tissue, represents an intermediate type of air chamber with regard to high specialization.

Marked degeneration of chloroplasts occurs in the outer cells of the thallus. As this degeneration progresses, the plastids break down into a mucilage-like substance which probably protects the plant from desiccation. After this disintegration of plastids, the walls of the outer cells are no longer distended by turgor, and they collapse and assume a more or less hemispherical or meniscus shape. Often adjacent cells of this row meet closely over the air chambers, closing them completely and retarding evaporation. In the older parts of the thallus the plastids in the layer of cells immediately below these show the beginnings of disintegration, and seem destined for the same fate as the outermost ones, upon the death of the latter (fig. 3).

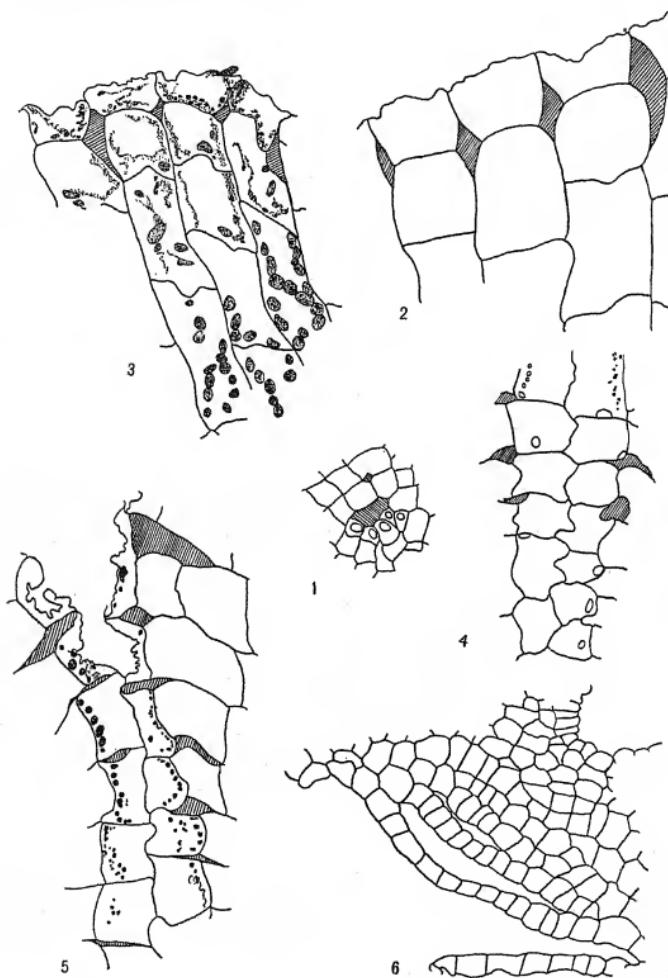
In the region of the sex organ groove, which is completely closed,

¹ Barnes, C. R., and Land, W. J. G., Bryological papers. I. The origin of air chambers. Bot. Gaz. 44: 197. 1907.

² Leitgeb, H., Untersuchungen über die Lebermoose. 4: 10. 1879.

³ Hirsch, Pauline, The development of air chambers in the Ricciaceae. Bull. Torr. Bot. Club 37: 73-77. 1910.

except at the front of the thallus, the cells forming the outer layer are large and rather thick walled. The outer cells of one wing are often fused with those of the other; in any case there is a very close



interlocking (figs. 4, 5). Probably this protects the delicate meristematic region and the developing sex organs from drying out. In all the preparations examined, the marginal cells at the base of the groove are turgid, and do not give the appearance of having been subjected to excessive lack of moisture. LAND reports that a slight amount of moisture was found in the groove in some plants cut open in the field.

The abundant scales, curving over and closely investing the meristematic region at the apex of the thallus, arise in the usual manner from surface cells on the lower side of the thallus. They probably function in preventing loss of moisture from the meristematic area and in affording protection from mechanical injury. These scales are shown in fig. 6.

As compared with the Marchantiaceae, the development of the mucilage hairs in these plants is very slight. This casts additional doubt on the correctness of the idea that these liverworts depend to as great a degree as some believe upon an abundance of mucilage to keep them from drying out in relatively arid situations.

In summarizing this study, therefore, it may be stated that structurally the thallus of this species of *Riccia* from an exceedingly arid Mexican mountain side possesses three features which are almost certainly of survival value in such an environment. They are (1) ventral scales which closely invest the growing point; (2) a tightly closed sex organ groove except at the front of the thallus; and (3) a row of superficial cells, the plastids of which break down into a mucilage-like substance, and the walls of which collapse upon the resulting lack of turgor, so that adjacent cells meet over the air spaces. Obviously these features not only serve to protect the plants possessing them from mechanical injury, but tend to conserve their moisture content, which in a dry situation is of major significance.

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BRIEFER ARTICLES

SHRINKAGE AND GROWTH IN PLANT STEMS

The retardation of the growth rate of stems during the day has frequently been noted, but the fact that there may be an entire cessation of growth, or even an actual shrinkage in length during the intense heat of midday or shortly thereafter, due to loss of water from the tissue of the stem through transpiration, is not so widely known. This paper records such an observation made on five different species of plants, four herbaceous and one woody.

BROWN and TRELEASE¹ record their observations on this, and also cite instances recorded in the literature. They observed at hourly intervals changes in the length of the stems of *Cestrum nocturnum*, a cultivated shrub, growing where it was exposed to severe conditions of light and temperature. They found a shrinkage of 0.5–2.5 mm. in the length of the stem during the middle of the day, and a recovery of the original length when the plant became shaded. The period of maximum shrinkage did not coincide with the period of greatest evaporation from a Livingston white spherical atmometer, but occurred several hours earlier. They believed that sunlight had a greater effect on the wilting of the plant and the shortening of the stem than the evaporating power of the air as measured by the white atmometer.

During the summer of 1924 observations were made on several plants whose runners stretched for many feet over the blazing white sand of the beach. The observations were made near Puerto Galera, Mindoro, Philippine Islands. It was a gently sloping area of fine white coral sand fully exposed to the sun from early morning until evening. The upper beach supported a sparse vegetation characteristic of the beaches of the Philippines. Many of the species of this beach vegetation send out prostrate stems or runners, which stretch over the sand for many feet, in the case of *Ipomoea Pes-caprae* for as much as 30 feet.

Measurements were made on five species, *Ipomoea Pes-caprae*, *Canavalia rosea*, *Wedelia biflora*, *Vigna marina*, and *Quisqualis indica*.

¹ Alternate shrinkage and elongation of growing stems of *Cestrum nocturnum*. Phil. Jour. Sci. 13: 353–360. 1918.

An ink mark was made on the older portion of the stem where growth had ceased (usually 80-120 cm. from the tip), and the growing stem measured from this point to the extreme tip. This was done at 6:30 P.M. on the previous evening. The measurements were begun in the morning at 6:00 A.M., and only those plants were used which showed a normal growth during the night. The measurements were continued at hourly intervals throughout the day until 6:00 P.M. The final measurement was made at 6:30 A.M. the following morning to determine whether normal growth had occurred during the night. In some cases the handling of the plant incident to the measuring injured the delicate growing tip, resulting in the blackening and wilting of the injured portion. The day on which the measurements were made was a remarkably clear, hot day, with a brilliant sun and little air movement.

TABLE I

GROWTH MEASUREMENTS ON STEMS (GROWTH INCREMENT OR DECREASE
OVER NEXT PRECEDING MEASUREMENT IN MM.)

PLANTS	APRIL 28	APRIL 29												APRIL 30	
		6:30 P.M.	6:00 A.M.	7:00 A.M.	8:00 A.M.	9:00 A.M.	10:00 A.M.	11:00 A.M.	12:00 A.M.	1:00 P.M.	2:00 P.M.	3:00 P.M.	4:00 P.M.		
<i>Ipomoea Pes-caprae</i>	o	67	3	o	2	1	o	-3	o	o	6	7	2	6	40
<i>Canavalia rosea</i>	o	12	o	1	o	o	o	o	-1	1	1	-1	o	o	3
<i>Wedelia biflora</i>	o	10	o	o	o	o	o	-2	-1	o	o	3	2	1	5
<i>Vigna marina</i>	o	21	o	o	1	1	o	-1	-4	o	-3	4	-2	o	Broken
<i>Quisqualis indica</i> ...	o	42	2	o	1	1	o	-1	o	o	2	3	5	o	19

The following reading of the environmental factors was made on a nearby beach, with the conditions of sand, vegetation, and sun similar to the beach where the measurements were taken. April 29, 1924: Livingston white spherical atmometer 41.6 cc. in 24 hours; this was one of the highest readings recorded during a period of over eight weeks. The air temperature was 84.5° F. at 5:00 P.M. No maximum was recorded during the day, but it was probably considerably above 90° F. in the shade (table I).

The growth during the night (from 6:30 P.M. to 6:00 A.M.) was considerable in all species and exceptional in *Ipomoea*, which increased 67 mm. in length. All of the plants showed very evident signs of wilting during the middle of the day. *Ipomoea* folded up its leaves with the upper side inward, while the leaflets of *Canavalia* and *Vigna* folded up in a similar manner and presented much less leaf surface to the sun and air. The leaves of *Wedelia* and *Quisqualis*, without these adaptations, became

very flaccid and wilted. They all recovered toward evening, beginning at about 3:00 P.M.

Ipomoea proved a very rapid grower, showing an increase in length up to 10:00 A.M., in spite of the great heat and water loss, and beginning its increase again at 3:00 P.M.; while during the night following the day of measurements it grew 46 mm. During the heat of midday it became 3 mm. shorter, and did not recover this shortage for several hours. The day's heat started early, resulting in almost no increase after 7:00 A.M. *Wedelia* grew none during the forenoon, shrunk 3 mm. at midday, and regained this from 4:00 P.M. on. The irregular record of *Vigna*, especially its shrinkage late in the afternoon, was undoubtedly due to injury while being measured, as became evident the next morning. In no case was the growth during the night following the measurements as great as during the night preceding them, probably due to disturbing the plant during measuring. The first elongation early in the afternoon was not true growth, but merely regaining the length which the stem had shrunken during midday. In accord with the finding of BROWN and TRELEASE, it was found that the greatest shrinkage occurred between 12:00 and 1:00 P.M., and hence did not coincide with the period of greatest evaporation from a Livingston white atmometer; also elongation began again at 2:00-3:00 P.M., when the rate of evaporation was still very high.—RAYMOND KIENHOLZ, *University of Illinois, Urbana, Ill.*

[Accepted for publication March 6, 1926]

CURRENT LITERATURE

BOOK REVIEWS

Manual of cultivated trees and shrubs

It is no small task to bring together a classified analytical critical description of nearly 2500 species, and as many differentiated varieties, representing over 400 genera and a quarter as many families. This is what REHDER has done in his manual of the woody plants known as hardy in North American parks and gardens.¹

REHDER is unsurpassed in knowledge and experience in this field. For a quarter of a century he has clarified for himself and others the characteristics of woody plants through succeeding editions of BAILEY'S *Cyclopedia*, in which he has treated the greater number of such plants. The *Manual* into which his long experience has ripened now is literally a handbook, light enough to be carried afield notwithstanding its thousand pages of text.

Special features of the *Manual* are a double-columned five-page explanation of abbreviations of authors' names; an extensive list of works to which reference is made for illustrations; explanation of geographic and miscellaneous abbreviations; metric and duodecimal measurement scales; and a map of North America divided into isothermic or hardness climatic zones. A synoptical key to natural orders and families claims five pages; and an analytical key to families and aberrant genera, based on customary taxonomic characters, occupies another five. The index to plant names, including extensive synonymy, occupies seventy closely set three-column pages, which may give an idea of the comprehensiveness of the book.

The descriptive contents of such a volume contrast noticeably with those of an ordinary guide to a regional flora, because on the one hand it is restricted to a fractional part of the families represented, and on the other hand it takes account of definable varieties outnumbering the species to which they pertain. For example, 29 genera of gymnosperms with 199 species are included, and monocotyledons are represented by only 9 genera and 56 species. Numerous families unknown in our native flora find representation, although usually in a small number of forms. As might be expected, the Schizopetalae stand out in prominence, with 60 families, to which half of the descriptive part of the book is devoted; the Gamopetalae with nearly half as many families (27) claim little more space than the single family Rosaceae; and the Apetalae with over half as

¹ REHDER, ALFRED, *Manual of cultivated trees and shrubs hardy in North America exclusive of the subtropical and warmer temperate regions.* 8vo. pp. xxxvii+930. New York: Macmillan Co. 1927.

many families (15) as the Gamopetalae average only two pages to the family, largely taken up by the oaks with 58 species. Among the most familiar and here outstanding families are the Caprifoliaceae (10 genera occupying nearly 50 pages); Oleaceae (11 genera, claiming only about half the space); Ericaceae (34 genera, about as extensively treated as the less varied Caprifoliaceae); Leguminosae (39 genera but averaging only a page to the genus); and, as would be expected, Rosaceae (48 genera, claiming nearly as much space as all of these other families together).

The author has been reasonably consistent in the matter of nomenclature, following international rules in the main, which will meet with warmer approval in some circles than in others. This will render comparison fairly easy with the *Cyclopedia of horticulture* and with one series of handbooks of our native flora, the former especially to be commended. Happily, intergeneric hybrids are designated by pseudo-generic names; and interspecific hybrids by pseudo-specific names, naturally with indication of accepted parentage. Minor forms are treated trinomially.

A request is modestly inserted for material and information that will enable later editions to be made better than the first. No doubt many improvements and additions will be brought to his attention; the author himself has added three pages of emendations. From the reviewer's viewpoint, no new feature is more desirable than the incorporation of keys based on summer and winter vegetative characters, because flowers are present for only a brief time each year if at all. For the preparation of such keys no more competent botanist lives than the author.—WM. TRELEASE.

Introduction to cytology

That a second edition of SHARP's book² should be needed so soon is proof that the work has had a wide range of usefulness. While WILSON's recent revision of his book on the cell has given considerable attention to plants, and is extremely valuable because it gives to botanists the interpretations of an able zoölogist, the botanist nevertheless needs a comprehensive treatment written primarily from the botanical standpoint. Naturally, this second edition has been improved and enlarged, and the author has doubtless profited by the advice and criticisms of those who have used the first edition. The number of pages and of illustrations has been increased, and also some of the previous figures which are retained have been improved.

Descriptions of mitosis, especially the reduction mitoses, have been thoroughly revised, and many of the new figures occur in this section. In regard to the structure of the chromosome, the author believes that a "chromonema" of some sort is probable, but its relation to the alveolized condition, seen so clearly in larger chromosomes, remains to be determined. The author suggests that this situation demands a further study of fixation. The reviewer would sug-

² SHARP, L. W., An introduction to cytology. 8vo. pp. xiv+581. figs. 210. New York: McGraw-Hill Book Co. 1926.

gest a more critical comparison of living and fixed material. The theory that the nuclear reticulum consists of a single substance (chromatin), rather than a series of chromomeres upon an achromatic linin, is discussed, and the "chromomeres" are interpreted as thick regions of the chromatic thread and not as chromomeres supported by another material.

In dealing with heredity, many changes have been necessitated by recent researches upon chromosomes in polyploid organisms and in hybrids. There is also new material on sex chromosomes and the relation of the chromosome theory to the metabolic theory of sex determination. There is a new chapter, with much new material, on gametogenesis, dealing with both plant and animal organisms. Chromosomes and also blepharoplasts receive attention here. In general, more attention is given to studies on living tissues, especially the achromatic figure and meiosis. Other sections which are more adequately treated in the second edition are those dealing with chromidia, chondriosomes, and cytokinesis.

It is evident that more attention has been given to LAMARCK and other French biologists in the development of ideas of the structure of organisms. The organismal theory is prominent in the histological treatment, especially in the discussion of the relation of cells to differentiation and other activities of protoplasm. The author seems dominated by the idea that the action of a system is not simply the sum of the action of its parts, but that the parts act as they do because of their relation to the rest of the system. Whether the theory is correct or not, it introduces an element of unity and affords a reasonable explanation of various phenomena.

In the new edition there is one very complete bibliography, arranged alphabetically, with a chronological grouping of the works of each author. This is a great improvement on the numerous fragmentary bibliographies of the first edition. The index is extensive and has catchwords and full face type for illustrations. It is hoped that the book will continue to improve with the rapid and multifarious development of the subject.—C. J. CHAMBERLAIN.

Anatomy of angiosperm seed

Another volume of the *Handbuch der Pflanzenanatomie*, under the general supervision of K. LINSBAUER, deals with the structure of the angiosperm seed. As projected, the series covers the whole field of the structure of plants, from the algae to the orchids, the various phases being assigned to various botanists. Since each part is published when the manuscript is ready, as in case of ENGLER'S *Pflanzenfamilien*, the parts do not appear in the sequence indicated by the general table of contents. In the complete table there are two series, general and special, and of these the first is subdivided into cytology, histology, and experimental anatomy; the rest of the work is also highly organized. Some idea of the scope of the subject and the standing of the men handling it may be gained from the titles of the five volumes devoted to cytology: *Cell and cytoplasm*, by LUNDEGÅRDH; *Plastiden*, by SCHÜRHoff; *Pflanzenkaryologie*, by

TISCHLER; *Cell materials*, by RICHTER, NETOLITZKY, and MÖBIUS; and *Cell membrane*, by WISSELINGH. The rest of the volumes are also by men of high rank. The whole series is a useful, well organized combination of compilation and investigation by experts.

Volume X is by NETOLITZKY, on the anatomy of the angiosperm seed.³ It includes much literature supplementing the scattered and incomplete accounts of this important structure. Unfortunately, QUISUMBING's investigation of the origin and development of the seed coat was overlooked, probably because the title of this work suggests that it deals only with gymnosperms.

The first part treats the ovule, integuments, nucellus, and embryo. Attention is given to the seed coat as an ecological entity, to the protective layers, and to the value of the seed coats in taxonomy and phylogeny. Most of the text and illustrations deal with the mature or nearly mature seed. In the second part the essential characters of the seed are given for most of the families, with citations of the more important literature closing the account of each family.

A very useful feature is a 13-page table, listing family by family some of the principal seed characters. Besides the usual bibliography, there is a second author index, giving the pages on which references are made to the work of each investigator. HOFMEISTER is referred to 65 times, and references to many others are numerous. There is an index to the genera of all the plants mentioned in the text, and also an extensive subject index.

This series of books, summaries of various phases of botany, largely editorial, but confirmed, corrected, and extended by experienced investigators in the different fields, should simplify and shorten the preliminary work of workers who need to know what has already been done in any particular subject. While the student would not be relieved of the necessity for consulting original sources, this series will at once put him into contact with most of the literature.—C. J. CHAMBERLAIN.

A New Zealand Manual

In 1906 CHEESEMAN published the first manual of the New Zealand flora, which was reviewed in this journal.⁴ Soon after its appearance the advance in knowledge of the flora of these islands made a revision necessary, and on this the author was still working when he died at Auckland, in 1923. From his manuscript, from extensive notes, and from his published papers the revision has been completed by OLIVER, who assures us that his contribution consists only in editing the material with as few changes as possible.

In this edition⁵ an addition of 192 species brings the total number to 1763,

³ NETOLITZKY, FRITZ, *Anatomie der Angiospermen-Samen*. 8vo. pp. v+364. figs. 550. Berlin: Gebrüder Bornträger. 1926.

⁴ BOT. GAZ. 42: 495. 1906.

⁵ CHEESEMAN, T. F., *Manual of the New Zealand flora*. 2d. ed. Edited by W. R. B. OLIVER. 8vo. pp. xliv+1163. Washington: W. A. G. Skinner, Government Printer. 1925.

comprising 159 pteridophytes, 20 gymnosperms, 400 monocotyledons, and 1184 dicotyledons. Five families possess over 100 species each. They are the Compositae with 261 species, the Filices with 141, the Scrophulariaceae with 138, and the Cyperaceae and the Gramineae with 123 species each. Some of the other large families, in order of size, are Umbelliferae, Orchidaceae, Ranunculaceae, Rubiaceae, Onagraceae, Epacridaceae, Boraginaceae, and Leguminosae. Among the genera with 20 or more species each are *Veronica*, *Celmisia*, *Coprosma*, *Olearia*, *Ranunculus*, *Cotula*, *Raukia*, *Carmichaelia*, *Hymenophyllum*, and *Dracophyllum*. The endemic element of the flora is conspicuously large, consisting of 1329 species, or over 75 per cent of the whole. Of the species found also in other lands, 369 extend to Australia and 112 occur in South America.

An important part of the volume, and one particularly interesting to those living outside New Zealand, is a history of the botanical discovery and exploration of the region. This dates back to COOK's first visit in 1769-70, on which he was accompanied by the celebrated naturalist Sir JOSEPH BANKS. On COOK's second voyage he was accompanied by JOHN R. FORSTER and his son GEORGE FORSTER, and also by Dr. SPARRMAN, a former pupil of LINNAEUS. All of these men, who were botanists of ability, had the privilege of exploring virgin territory and attracting others to complete the work. In the present volume OLIVER has brought the botanical history of New Zealand down to 1924.

In addition to the main part of the manual, containing the usual keys and descriptions of genera and species, the volume contains a list of plants naturalized in New Zealand, an alphabetical list of Maori names of plants, a glossary, and a list of the published works of T. F. CHEESEMAN.—G. D. FULLER.

NOTES FOR STUDENTS

Relation of iron to chlorophyll development in growth of plants.—Iron is one of the essential elements for the growth of plants, because it is needed for chlorophyll development, but it is not known why it is needed for this process. A few years ago POLACCI and ODDO advanced the theory, based upon their own experiments and the work of WILLSTÄTTER on the chemistry of chlorophyll, that iron catalyzes the formation of pyrrole groupings which enter into the composition of the nucleus of the chlorophyll molecule. These workers state, however, that if pyrrole groups of a kind that the plant can use are supplied, then iron becomes unnecessary for the development of chlorophyll. They described experiments in which normal green plants were obtained, when suitable pyrrole groups but no iron were supplied.

DEUBER⁶ has been unable to confirm the work of POLACCI and ODDO. Even in weak concentrations the pyrrole salt proved toxic and did not prevent chlorosis when iron was absent. All possibilities which may explain these divergent results were eliminated by experiments, except the factors having to do with the

⁶ DEUBER, C. G., Can a pyrrole derivative be substituted for iron in the growth of plants. Amer. Jour. Bot. 13:276-285. 1926.

differences in environment, but that the differences in the results obtained were not due to different environmental conditions was strongly indicated by limited experiments with a sample of the pyrrole salt used by POLACCI and ODDO in their experiments. This salt also proved toxic and did not prevent chlorosis when iron was absent.

It would seem that the question of the relation of iron to chlorophyll development remains yet to be solved.—S. V. EATON.

Ancient lotus fruits.—OHGA⁷ has investigated the structural conditions of dormancy in some fruits of Indian lotus (*Nelumbo nucifera*), said to be at least several centuries old, which proved to be still viable under certain conditions. In a previous paper, published in 1923, the announcement of their discovery was made. In the present paper the structural details are presented. The statement is made that "they appear to be the oldest viable seeds thus far known." The fruits were found in a prehistoric peat bed in South Manchuria. More than 200 of these ancient fruits were tested for viability and all of them germinated. A detailed account of their structure is presented, and the conclusion reached that their prolonged viability is due to their burial in the soil and their hard coats, which are impermeable to water. Experiments showed that water cannot enter unless the fruit coat is removed or broken up by some mechanical or chemical treatment. The most satisfactory solvent was found to be sulphuric acid. Even when treated for 24 hours with concentrated sulphuric acid, the embryo was not killed.—J. M. C.

Gametophyte and embryo of *Asplenium*.—FREER⁸ has investigated *Asplenium angustifolium*, a species of wide range, but apparently disappearing. The spores germinate slowly, requiring ordinarily about a month. The apical cell formation differs from the usual method in this group of ferns, the first division of the terminal cell being longitudinal instead of oblique, and both of the resulting cells dividing transversely. The protonemal filament is usually only three or four cells long when the terminal cell divides. The gametophyte is regularly heart-shaped. The archegonium develops as usual, with two exceptions: the first division of the inner cell sometimes precedes and sometimes follows the formation of the "chimney foundation from the cap cells"; the division of the central cell may precede or may follow that of the basal cell.—J. M. C.

Longevity of pollen.—HOLMAN and Miss BRUBAKER⁹ have conducted some extensive experiments on the longevity of pollen, testing 50 species of angio-

⁷ OHGA, ICHIRO, On the structure of some ancient, but still viable fruits of Indian lotus, with special reference to their prolonged dormancy. Japanese Jour. Bot. 3:1-20. 1926.

⁸ FREER, R. S., Notes on the development of the gametophyte and embryo of *Asplenium angustifolium* Michx. Ohio Jour. Sci. 26:147-168. 1926.

⁹ HOLMAN, R. M., and BRUBAKER, FLORENCE, On the longevity of pollen. Univ. Calif. Publ. Bot. 12:179-204. 1926.

sperms. Stored in low humidity the percentages of germination fell off suddenly after a few days or weeks, and then for a long period continued at a constant low rate. Stored air-dry most of the pollens survived for one to several weeks. The mean longevity when stored air-dry was about 23 days. The maximum longevity obtained was 336 days for *Typha latifolia*. Of nine families investigated, the Primulaceae ranked first in longevity, followed in order by Leguminosae, Saxifragaceae, and Rosaceae, while the Scrophulariaceae ranked last. The Gramineae, as has been known, stand far below any other family in pollen longevity.—J. M. C.

Cretaceous plants of Western Greenland.—SEWARD¹⁰ has published the results of his investigation of the Cretaceous plant-bearing rocks of Western Greenland, where he spent over two months in the summer of 1921. The plants collected include 15 Filicales, 8 Cycadophytes, 5 Ginkgoales, 19 Coniferales, and 18 Angiosperms. In addition to a general account of the geology of the region, and a full description of the plant material collected, SEWARD presents important conclusions as to the relation of the Cretaceous vegetation of Greenland with floras of other regions, the problem of geological age, and the Cretaceous climate. It is a valuable document for students of the evolution and distribution of plants throughout the northern regions.—J. M. C.

Thelephoraceae.—BURT¹¹ has completed his extensive monograph of the North American Thelephoraceae. The final paper is mainly the presentation of *Corticium*, which contains 108 species, 47 of which are described as new. A supplement includes species received since the publication of the earlier parts, 20 new species being described under 10 genera. The monograph is very detailed, not only in its description, but also in the complete record of specimens collected. The work as a whole includes descriptions of 113 new species, and the total number of species included in this family will be a revelation to those unfamiliar with the group.—J. M. C.

Micronitrogen determination.—Some improvements of apparatus for the determination of very small amounts of nitrogen are described by FUCHS,¹² by means of which he has reduced the error of determination to 0.007 mg. The apparatus is figured, but the manufacture is protected by law, and it can be obtained only from Alois Schmidt, Schuhbrücke, 42, Breslau. All rubber connections have been eliminated in the shaking apparatus. Complete description of the use of the apparatus is given.—C. A. SHULL.

North American Flora.—The eleventh part of volume VII contains a continuation of additions and corrections to Uredinales.—J. M. C.

¹⁰ SEWARD, A. C., The Cretaceous plant-bearing rocks of Western Greenland. Phil. Trans. Royal Soc. London 215:57-175. pls. 4-12. 1926.

¹¹ BURT, E. A., The Thelephoraceae of North America. XV. Ann. Mo. Bot. Gard. 13:173-354. 1926.

¹² FUCHS, H. J., Zwei verbesserte Apparate zur Mikrostickstoffbestimmung. Biochem. Zschr. 176:32-37. 1926.

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VIRULENCE, SEROLOGICAL, AND OTHER PHYSIOLOGICAL STUDIES OF BACTERIUM FLACCUMFACIENS,
BACT. PHASEOLI, AND BACT. PHASEOLI SOJENSE¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 364

C. G. SHARP

(WITH PLATE VII AND EIGHT FIGURES)

Introduction

It is a moot question among phytopathologists whether the organisms causing wilt of bean, *Bact. flaccumfaciens*, blight of bean, *Bact. phaseoli*, and pustule of soy bean, *Bact. phaseoli sojense*, deserve species rank or should be considered subspecies or varieties. *Bact. phaseoli* was described by SMITH (24) in 1897. He (25) describes the symptoms of the disease caused by this organism as follows, stressing the localized lesions in the leaf and stem parenchyma:

This is a disease of beans common on leaves, stems and pods, and confined principally to the parenchyma although the vessels also are involved, sometimes for a distance of several inches.

BURKHOLDER (2) studied bacterial blight of beans, which he describes as a systemic disease caused by *Bact. phaesoli*. He discussed two sets of symptoms, one characterized by lesions on the leaves, stems, and pods as described by SMITH, and commonly known as blight; and a second, unusual systemic symptom, characterized by the presence of the pathogene in the vascular bundles.

¹ Contribution from the department of botany and the department of hygiene and bacteriology, the University of Chicago.

MISS HEDGES (9) described a new organism which she had isolated from diseased beans. When inoculated into beans by pricks this organism produced wilt; it also differed from *Bact. phaseoli* culturally, and consequently she named it *Bact. flaccumfaciens*. Another paper by Miss HEDGES (13) on a comparative study of *Bact. flaccumfaciens* and *Bact. phaseoli* reported physiological differences in culture media, and differences in staining reaction, and pointed out that *Bact. flaccumfaciens* is a vascular invader, while *Bact. phaseoli* seldom is.

The organism *Bact. phaseoli sojense*, which causes bacterial pustule of soy bean, was described by Miss HEDGES (10) in 1922. In 1924 she (11) published a paper on the comparative study of *Bact. phaseoli* and *Bact. phaseoli sojense*, in which she reports that these organisms are different in their pathogenicity for plants (garden bean and soy bean), but that in culture media they are identical. She did, however, in some instances find a morphological difference in the kind of colonies produced, some colonies of *Bact. phaseoli sojense* on thinly sown plates having internal convolutions which were not found in colonies of *Bact. phaseoli*. WOLF (26, 27) concluded that *Bact. phaseoli sojense* is morphologically and culturally indistinguishable from *Bact. phaseoli*. It appears, therefore, that *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* are very closely related and are not well differentiated, *Bact. flaccumfaciens* standing most apart. The present investigation was undertaken to determine whether these organisms can be differentiated serologically. In the course of the investigation other problems arose, which necessitated a study of (1) the morphology of colonies and of some of the organisms; (2) the physiology of the organisms, specifically their action on various culture media such as milk, starch, gelatin, and sugars; (3) the virulence of the organisms; and (4) the phenomenon of acid agglutination of the organisms.

The cultures used in this study were very kindly furnished by Miss HEDGES, U.S. Department of Agriculture, Washington, D.C.

I. Morphological

Agar shake dilutions were made and plates poured to ascertain whether the cultures were free from contamination and to obtain

single colonies for study. From each culture were isolated two subcultures which were designated as *Bact. flaccumfaciens* no. 7 and no. 8, *Bact. phaseoli* no. 1 and no. 2, and *Bact. phaseoli sojense* smooth (*S*) and rough (*R*) respectively. The last two strains proved to be exceedingly interesting, especially in view of the fact that they came from an agar slant culture which macroscopically appeared smooth.

DESCRIPTION OF COLONIES

Bact. flaccumfaciens no. 7 produced very small round, raised colonies, which were rather dry in consistency, and which did not spread over the medium. *Bact. flaccumfaciens* no. 8 produced very thin flat colonies which were wet and sticky in consistency, and which had a tendency to spread over the medium. On agar slants no. 8 always spread over the surface in a very thin mucoid fashion, most of it settling to the bottom of the slant; no. 7 always remained dry in appearance. Because of these morphological differences, both of these strains were carried through all experiments.

Bact. phaseoli no. 1 was isolated from a colony characterized by a hyaline border and concentric rings (figs. 9, 10). On agar slants this culture appeared smoother than no. 2, and had a tendency to settle to the bottom of the slant. *Bact. phaseoli* no. 2 came from a regular raised colony without a hyaline border and without concentric rings (fig. 11).

Bact. phaseoli sojense (*S*) came from a round, regular, smooth colony which was very flat and which had a tendency to spread (fig. 12). *Bact. phaseoli sojense* (*R*) came from a colony which was raised and round, and which became umbonate and wrinkled or convoluted in 5-8 days. These colonies were very uneven on the surface (figs. 13-15). A histological study of cross-sections of the rough colony indicates that the irregularities and wrinkles are on the surface of the colony and not within it, as described by Miss HEDGES (11) for the aberrant colonies noted by her. This phase of the problem is under investigation at present. The colonies of the rough strain, instead of spreading over the medium as does the smooth, grow vertically. Agar slants made from the *R* culture were always rough and much more raised than those from the *S* strain. Both the *S* and *R* strains remained constant on culture media throughout the many

transfers made; however, when a pure line of the *R* strain was inoculated into soy bean plants, both *S* and *R* strains were obtained upon reisolations from the lesions. No change of the *S* to the *R* strain was ever obtained when the former was inoculated into plants. The *S* strain was found to be motile while the *R* strain is non-motile. These observations agree with the findings relative to *S* and *R* forms of certain animal pathogens.

Bact. flaccumfaciens no. 7 and no. 8 were both gram positive, while all the other organisms studied were gram negative.

Inoculation experiments proved that each of the subcultures of *Bact. flaccumfaciens* and *Bact. phaseoli* was pathogenic to the garden bean, and that *Bact. phaseoli sojense S* and *R* were pathogenic for the soy bean.

II. Physiological

Culture media consisting of litmus milk, 0.2 per cent starch agar, 2 per cent plain gelatin, and various sugar broths were used in these studies.

LITMUS MILK.—*Bact. flaccumfaciens* no. 7 and no. 8 both formed acid in 4 days, and caused a reduction of litmus in 25 days. The color of the litmus could be restored by alkali. No coagulation took place in 25 days, but the milk was almost completely peptonized. Neither *Bact. phaseoli* no. 1 and no. 2, nor *Bact. phaseoli sojense S* and *R* produced acid or reduced the litmus in 25 days. More rapid digestion of the milk was produced by the *S* and *R* strains, but all four completely digested milk in 25 days.

STARCH AGAR 0.2 per cent.—Both strains of *Bact. flaccumfaciens* hydrolyzed starch very slowly (fig. 16), while *Bact. phaseoli* no. 1 and no. 2 and *Bact. phaseoli sojense S* and *R* hydrolyzed it rapidly and equally (fig. 17).

PLAIN GELATIN 2 per cent.—The method used here in studying liquefaction of gelatin was that described by LEVINE and CARPENTER (18, 19). Twenty gm. of gelatin was added to 1000 cc. of water at 40° C. The material was then incubated at 41° C. for 4 hours, after which the reaction was adjusted to $P_{H_2} 7.2$. Twelve cc. of the medium was now placed in each of a series of test tubes and sterilized in the autoclave.

To compare quantitatively the amount of liquefaction produced in gelatin by *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* the following experiment was performed. Gelatin cultures were made and permitted to grow for 2 days at 26° C., the gelatin remaining liquid at this temperature. At the end of this period 4 cc. of each of the living cultures was removed aseptically from each tube, 0.5 cc. of which was inoculated into each of eight gelatin tubes. To the remainder of each of the old gelatin cultures was added 1 cc. of chloroform. These were allowed to stand for 4 hours, after which 1 cc. of each of these killed cultures was added to each of eight gelatin tubes. Formol titrations were next made on the 2nd, 5th, 10th, and 25th days. This was done by placing 5 cc. of the gelatin culture in an evaporating dish, adding 20 cc. distilled water, and neutralizing to phenolphthalein; 10 cc. of 50 per cent formalin (neutral to phenolphthalein) was then added, the mixture allowed to stand for 10 minutes, after which it was titrated with N/50 NaOH. The findings are recorded in table I. The results are essentially in accord with those obtained by LEVINE and CARPENTER (18, 19), in their work on living and phenolated cultures, and by KENDALL and his co-workers (17), on studies with *Proteus*. These investigators state that the slight liquefaction in phenolated tubes (since no growth in them was ever observed) must have been due to the enzymes secreted by the organisms. The conclusion from the data presented in table I is that *Bact. phaseoli* and *Bact. phaseoli sojense* are both rapid liquefiers of gelatin, and cannot be differentiated by this method. On the other hand, *Bact. flaccumfaciens* liquefies gelatin very slowly, and can therefore be differentiated from the other two by this method.

SUGARS.—Six sugars (dextrose, galactose, levulose, lactose, maltose, and sucrose) were used. Tubes containing 8 cc. of standard beef extract-peptone broth, P_H 7.2, were prepared and sterilized in the autoclave. Next 20 per cent solutions of each of the sugars used were prepared and sterilized by filtration; 2 cc. of each was then transferred aseptically to a large number of the tubes containing plain broth, thus making a 4 per cent sugar broth. These sugar media were allowed to incubate for 4 days before use to prove sterility. Control tubes (uninoculated) were carried throughout the experiment.

In each experiment duplicate and often triplicate tests were made and the average P_H for these taken. This was determined colorimetrically, using different indicators to check the accuracy of the

TABLE I

EFFECT OF PRESENCE OF ORGANISMS STUDIED ON FORMOL TITRATION
OF GELATIN WHEN CHLOROFORMED AND NOT CHLOROFORMED,
AND ON FINAL P_H

ORGANISM	DAYS INCUBATED AT 50° C.	cc. N/1 NaOH PER 100 CC. GELATIN						GELATIN		
		Not chloroformed			Chloroformed			Control (P_H)	Not chloroformed (P_H)	Chloroformed (P_H)
		Control gelatin	Organism added	Increase over control	Control gelatin	Organism added	Increase over control			
Bact. phaseoli no. 1	2	0.56	0.80	0.30	0.56	0.64	0.08	6.6	7.8	6.4
	5	0.40	0.96	0.56	0.40	0.48	0.08			
	10	0.56	2.28	1.72	0.56	0.68	0.12			
	25	0.60	3.84	8.24	0.60	0.96	0.36			
Bact. phaseoli no. 2	2	0.56	0.96	0.40	0.56	0.72	0.16	6.6	7.6	6.5
	5	0.40	1.72	1.32	0.40	0.64	0.24			
	10	0.56	4.04	3.48	0.56	1.04	0.48			
	25	0.60	9.72	9.12	0.60	1.64	1.04			
Bact. phaseoli sojense S	2	0.56	0.68	0.12	0.56	0.64	0.08	6.6	7.6	6.5
	5	0.40	1.08	0.68	0.40	0.52	0.12			
	10	0.56	4.08	3.52	0.56	0.72	0.16			
	25	0.60	9.92	9.32	0.60	1.04	0.44			
Bact. phaseoli sojense R	2	0.56	0.76	0.20	0.56	0.64	0.08	6.6	7.6	6.4
	5	0.40	0.96	0.56	0.40	0.52	0.12			
	10	0.56	3.80	2.24	0.56	0.76	0.20			
	25	0.60	9.44	8.84	0.60	1.04	0.44			
Bact. flaccumfaciens no. 7	2	0.56	0.56	0.00	0.56	0.56	0.00	6.6	7.0	6.5
	5	0.40	0.40	0.00	0.40	0.40	0.00			
	10	0.56	0.72	0.16	0.56	0.56	0.00			
	25	0.60	1.64	1.04	0.60	0.60	0.00			
Bact. flaccumfaciens no. 8	2	0.56	0.58	0.02	0.56	0.58	0.02	6.6	6.8	6.5
	5	0.40	0.40	0.00	0.40	0.40	0.00			
	10	0.56	0.72	0.16	0.56	0.58	0.02			
	25	0.60	0.84	0.24	0.60	0.60	0.00			

work. The results of this experiment are recorded in table II. From these data it is seen that *Bact. flaccumfaciens* produced acid in all the sugars used, and thus can be differentiated from the other two organisms. *Bact. phaseoli* and *Bact. phaseoli sojense* produced little

TABLE II
CHANGE IN H-ION CONCENTRATION OF VARIOUS SUGAR MEDIA BY BACT. FLACCUMFACENS,
BACT. PHASEOLI, AND BACT. PHASEOLI SOJENSE

ORGANISM	DEXTROSE P_H 7.2*						GALACTOSE P_H 7.1*						LEVULOSE P_H 6.9*					
	P_H after no. of days						P_H after no. of days						P_H after no. of days					
	5	10	20	30	40	5	10	20	30	40	5	10	20	30	40	5	10	20
Control	7.2	7.2	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	6.9	6.8	6.8	6.8	6.8	6.8	6.8	6.8
Bact. phaseoli no. 1...	7.2	7.4	7.3	7.0	6.8	7.1	7.2	6.7	6.6	6.4	7.1	6.8	6.6	6.2	7.2	7.0	6.8	6.8
Bact. phaseoli no. 2...	7.3	7.4	7.3	7.3	7.5	7.2	7.1	6.8	6.8	6.4	7.1	7.2	6.8	7.0	7.0	7.3	7.4	7.4
Bact. phaseoli sojense S...	7.3	7.4	7.4	7.5	7.5	7.2	7.1	7.0	6.6	6.6	7.2	7.3	7.3	7.3	7.4	7.4	7.4	7.4
Bact. phaseoli sojense R...	7.3	7.4	7.3	7.2	6.9	7.2	7.0	6.8	6.4	6.0	7.2	7.4	7.4	7.4	7.4	7.4	7.4	7.4
Bact. flaccumfaciens 7...	7.0	6.9	6.6	4.6	4.0	7.0	6.6	5.2	4.8	4.8	6.3	5.2	5.0	4.8	4.8	4.8	4.8	4.8
Bact. flaccumfaciens 8...	7.0	6.8	5.7	4.6	4.6	7.1	6.7	6.3	5.2	5.2	6.2	5.3	5.1	4.8	4.8	4.8	4.8	4.8
LACTOSE P_H 7.1*																		
Control	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7.1	7.1	7.3	7.3	7.3	7.2	7.2	7.0	6.8	7.0
Bact. phaseoli no. 1...	7.4	7.4	7.5	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.8	7.4	7.2	7.0	6.8	7.0	6.8	7.0
Bact. phaseoli no. 2...	7.3	7.2	7.2	7.1	6.6	7.2	7.2	7.2	7.2	7.1	7.1	7.4	7.3	7.2	7.1	6.6	7.1	6.6
Bact. phaseoli sojense S...	7.6	7.7	7.9	8.2	8.2	8.2	7.4	7.4	7.4	7.2	7.3	7.4	7.4	7.4	7.3	7.3	7.3	7.3
Bact. phaseoli sojense R...	7.6	7.7	7.9	8.2	8.2	8.2	7.4	7.2	7.2	7.1	7.4	7.4	7.4	7.4	7.4	7.1	6.6	7.1
Bact. flaccumfaciens 7...	7.1	6.9	6.6	5.7	5.2	7.2	6.7	5.6	4.6	4.6	7.1	6.4	5.4	4.8	4.6	4.6	4.6	4.6
Bact. flaccumfaciens 8...	7.1	6.9	6.2	5.7	5.7	7.2	6.8	5.8	5.5	5.5	7.2	6.8	5.8	4.9	4.9	4.8	4.8	4.8
MALTOSE P_H 7.1*																		
Control	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.3	7.4	7.4	7.2	7.0	6.8	7.0	7.2
Bact. phaseoli no. 1...	7.4	7.4	7.5	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.8	7.4	7.2	7.0	6.8	7.0	6.8	7.0
Bact. phaseoli no. 2...	7.3	7.2	7.2	7.1	6.6	7.2	7.2	7.2	7.2	7.1	7.1	7.4	7.3	7.2	7.1	6.6	7.1	6.6
Bact. phaseoli sojense S...	7.6	7.7	7.9	8.2	8.2	8.2	7.4	7.4	7.4	7.2	7.3	7.4	7.4	7.4	7.3	7.3	7.3	7.3
Bact. phaseoli sojense R...	7.6	7.7	7.9	8.2	8.2	8.2	7.4	7.2	7.2	7.1	7.4	7.4	7.4	7.4	7.4	7.1	6.6	7.1
Bact. flaccumfaciens 7...	7.1	6.9	6.6	5.7	5.2	7.2	6.7	5.6	4.6	4.6	7.1	6.4	5.4	4.8	4.6	4.6	4.6	4.6
Bact. flaccumfaciens 8...	7.1	6.9	6.2	5.7	5.7	7.2	6.8	5.8	5.5	5.5	7.2	6.8	5.8	4.9	4.9	4.8	4.8	4.8
Sucrose P_H 7.3*																		

* Original P_H of media.

change in 30 days in dextrose, maltose, and sucrose, but formed acid in galactose. *Bact. phaseoli* in 30 days changed levulose from P_H 6.8 to 6.2-6.8, and lactose from P_H 7.1 to 7.6; while *Bact. phaseoli sojense* changed levulose to P_H 7.3 and lactose to P_H 8.2. *Bact. phaseoli sojense S* produces a very scaly pellicle which is very characteristic on lactose and levulose, and resembles duckweed on a pond. *B. phaseoli sojense R* in most of these sugars produced a very heavy granular precipitate.

The conclusion drawn from these data is that since *Bact. phaseoli* no. 1 and no. 2 did not always act alike, producing at times different quantities of acid and alkali in various sugars, it would be unwise to assert that the differences obtained between *Bact. phaseoli* and *Bact. phaseoli sojense* are sufficient to differentiate them. It is possible that other strains of each of these organisms might act exactly alike on these sugars. The alkalinity produced in levulose and lactose by *Bact. phaseoli* and *Bact. phaseoli sojense* is probably due to their hydrolytic action on the proteins in the broth yielding products with alkaline reactions, and not to any change occurring in the sugars.

III. Serological

A. AGGLUTININ TEST

For the serological differentiation of *Bact. flaccumfaciens* no. 7 and no. 8, *Bact. phaseoli* no. 1 and no. 2, and *Bact. phaseoli sojense S* and *R* the agglutination and precipitation tests were used. The agglutinin test, because of its partial specificity, has become one of the most important methods for the diagnosis of various animal diseases and for the classification of animal pathogens. Heretofore little use has been made of it in the field of plant pathology for the diagnosis of disease or identification of pathogens. The literature for this subject is reviewed in the accompanying paper by LINK and SHARP.

METHOD OF PROCEDURE.—After all the subcultures under consideration had been proved pathogenic to bean plants, each culture was grown on standard potato dextrose agar slants (P_H 7.2) for 4 days. The slants were then washed off with 0.85 per cent salt solution, and an even suspension of each organism was made to contain

TABLE III
AGGLUTINATION TESTS USING BACT. FLACCUMFACTENS NO. 7 ANTISERUM
AGAINST SUSPENSIONS

DILUTION OF SERUM	BACT. FLACCUMFACTENS NO. 7			BACT. FLACCUMFACTENS NO. 8			BACT. PHASEOLI NO. 1			BACT. PHASEOLI NO. 2		
	Tests			Tests			Tests			Tests		
	1	2	3	1	2	3	1	2	1	2	1	2
1:5.....	+	++	+++*	-	-	-	-	-	-	-	-	-
1:10.....	++*	+++	++++	-	-	-	-	-	-	-	-	-
1:20.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:40.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:80.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:160.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:320.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:640.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:1280.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:2560.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
1:5120.....	+++*	++++	++++	-	-	-	-	-	-	-	-	-
Saline.....	-	-	-	-	-	-	-	-	-	-	-	-

* Slightly less than the number of pluses indicated.

† In this and all the following tables where the symbols are used, ++++ stands for complete agglutination, and +++, ++, +, and - stand respectively for three-fourths, one-half, and one-fourth complete agglutination.

TABLE IV
AGGLUTINATION TESTS USING BACT. PHASEOLI NO. I
ANTISERUM AGAINST SUSPENSIONS

DILUTION OF SERUM	BACT. PHASEOLI NO. I			BACT. PHASEOLI NO. 2			BACT. PHASEOLI SOJENSE S			BACT. FLACCIDUM-CHIENS NO. 7			BACT. FLACCIDUM-CHIENS NO. 8		
	Tests			Tests			Tests			Tests			Tests		
	x	2	3	x	2	3	x	2	3	x	2	3	x	2	3
I:5.....	+	+	+	+	+	+	+	++*	++*	+	+	+	-	-	-
I:10.....	+	+	+	+	+	+	+	++*	++*	-	-	-	-	-	-
I:20.....	+	+	+	+	+	+	+	++*	++*	-	-	-	-	-	-
I:40.....	+	+	+	+	+	+	+	++*	++*	-	-	-	-	-	-
I:80.....	+	+	+	+	+	+	+	++*	++*	-	-	-	-	-	-
I:160.....	+	+	+	+	+	+	+	++*	++*	-	-	-	-	-	-
I:320.....	+	+	+	+	+	+	+	++*	++*	-	-	-	-	-	-
I:640.....	+	+	+	+	+	+	+	++*	++*	-	-	-	-	-	-
I:1280.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I:2560.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I:5120.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Slightly less than the number of pluses indicated.

TABLE V
AGGLUTINATION TESTS USING BACT. PHASEOLI SOJENSE S ANTISERUM
AGAINST SUSPENSIONS

DILUTION OF SERUM	BACT. PHASEOLI SOJENSE S			BACT. PHASEOLI NO. 1			BACT. PHASEOLI NO. 2			BACT. PHACCUMA-CIENS NO. 7			BACT. PHACCUMA-CIENS NO. 8		
	Tests			Tests			Tests			Tests			Tests		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
I:5.....	++	++	++	++*	++	+	++*	+	+	++*	+	+	++*	+	++
I:10.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:20.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:40.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:80.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:160.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:320.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:640.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:1280.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:2560.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
I:5120.....	++	++	++	++	++	-	++	-	-	-	-	-	-	-	-
Saline.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Slightly less than the number of pluses indicated.

approximately two billion organisms per cc. Next the suspensions were heated for 1 hour at 60° C. to kill the organisms. These suspensions were then used as the stock antigen.

Five intraperitoneal injections were made into rabbits at 4-day intervals, using 50 million dead organisms for the first injection, 100 million for the second, and 200 million for each of the last three injections. Five days after the last injection the rabbits were bled and the serum prepared. Agglutination tests were now set up, using

TABLE VI
AGGLUTINATION TESTS USING NORMAL SERUM AS CONTROL AGAINST SUSPENSIONS

DILUTION OF SERUM	BACT. PHASEOLI SOJENSE S			BACT. PHASEOLI NO. 1			BACT. PHASEOLI NO. 2			BACT. PLACCUMFA- CIENS NO. 7			BACT. PLACCUMFA- CIENS NO. 8		
	Tests			Tests			Tests			Tests			Tests		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1:5.....	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
1:10.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:20.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:40.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:80.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:160.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:320.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:640.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:1280.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:2560.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:5120.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saline.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

the serum prepared from each organism with its homologous antigen as well as with the other antigens, but no agglutination was obtained. This may mean that when they were killed by heat: (1) the antigenic properties of the organisms were destroyed; (2) the doses used were too small or too few; or (3) both of these factors were involved.

Heavy suspensions of the living organisms were next prepared, and four intravenous injections were made into the ear of each of the rabbits at intervals of 4 days, using 0.5, 0.75, 1, and 1 cc. respectively. Twelve days after the last injection the rabbits were again bled, serum prepared, and agglutination tests set up.²

² For method of setting up the tests, and a typical protocol, see accompanying paper by LINK and SHARP.

The organisms used for the suspensions in the agglutination tests were grown on standard beef extract-peptone-dextrose agar. The bacteria were washed off in 0.85 per cent salt solution, washed twice by centrifuging, and were re-suspended after the second washing in salt solution, the last suspension being used for the agglutination tests. The organisms were grown on different media and were washed

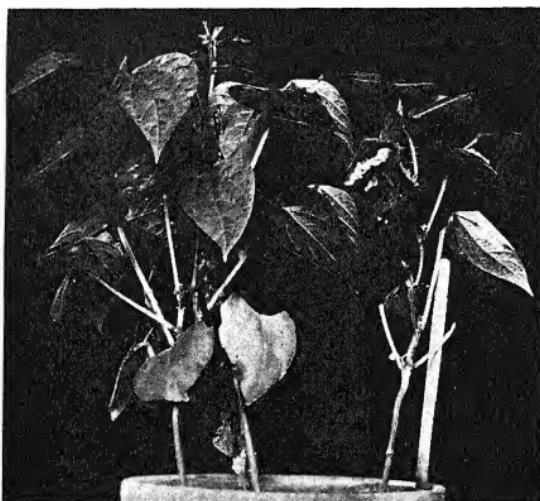


FIG. 1.—Bean plants 21 days after inoculation with *B. flaccumfaciens* no. 7; only slight infection present.

in order to prevent any possible chance of a precipitin reaction with the protein from the potato agar used for preparing the antigen.

The protocols of the experiments are given in tables III–VI. Table III shows that the antiserum of *Bact. flaccumfaciens* no. 7 gives specific agglutination with its own homologous strain and with no. 8, but that it fails entirely to agglutinate *Bact. phaseoli* no. 1 or no. 2 and *Bact. phaseoli sojense S*. No explanation can be given for not obtaining agglutination in test 1 against *Bact. flaccumfaciens* no. 8.

The results in test 1 (tables III–V) were obtained with serum

prepared from the second bleeding of the rabbits 12 days after the last injection. Since there was not enough serum left to recheck the experiment, a third bleeding was made 10 days after the second bleeding, and tests 2 and 3 made with the last lot of serum obtained. No injection of antigen was made between the second and third bleeding.

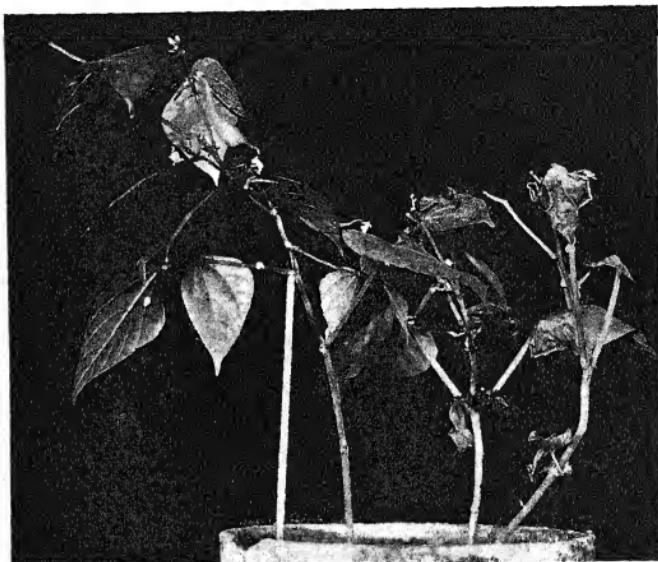


FIG. 2.—Same bean plants 21 days after inoculation with *B. flaccumfaciens* no. 8; plant not inoculated not infected, others nearly dead.

In all the tables the serum used in test 1 showed a higher titre than that used for tests 2 and 3. This indicates that the bleeding of the rabbits or the time elapsing after the last injection of antigen caused a reduction in the titre of all the sera used. Another very interesting phenomenon noted in all the tests reported in table III was that of the zone phenomenon. In dilution of 1-5 little agglutination occurred, while in dilutions 1-40 to 1-80 of tests 1 and 2, and 1-20 to 1-40 of test 3 there was almost complete agglutination in every case. Antiserum from strain no. 8 of *Bact. flaccumfaciens* was also used,

and it gave practically the same results as that obtained with antiserum from strain no. 7; therefore the conclusions are that antiserum from *Bact. flaccumfaciens* no. 7 and no. 8 will not serve to differentiate these two strains, but will differentiate *Bact. flaccumfaciens* from *Bact. phaseoli* and from *Bact. phaseoli sojense S.*

The data in table IV show that the antiserum from *Bact. phaseoli* no. 1 will differentiate the two strains no. 1 and no. 2 from *Bact.*

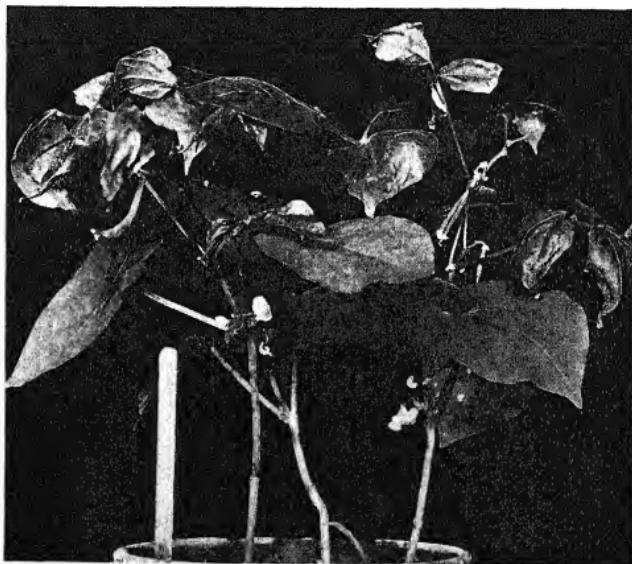


FIG. 3.—Bean plants 21 days after inoculation with *B. phaseoli* no. 1; extensive lesions present.

phaseoli sojense S., and *Bact. flaccumfaciens* no. 7 and no. 8. It also shows a slight group agglutination with *Bact. phaseoli sojense S* which may mean, according to current serological interpretation, that *Bact. phaseoli* contains a protein capable of stimulating slight antibody production against *Bact. phaseoli sojense S.*

The antiserum from *Bact. phaseoli* no. 2 acted in practically the same manner as the antiserum from no. 1, except that it failed to give quite as much group agglutination with *Bact. phaseoli sojense S*;

hence the two strains of *Bact. phaseoli* are identical serologically, but differ from the other two organisms. Practically no agglutination was obtained with *B. flaccumfaciens* no. 7 and no. 8, which indicates that, serologically at least, *Bact. phaseoli* and *B. phaseoli sojense* are more closely related than they are to *B. flaccumfaciens*. This is in harmony with the results obtained in the physiological studies reported, in which it was shown that *Bact. phaseoli* and *Bact. phaseoli*

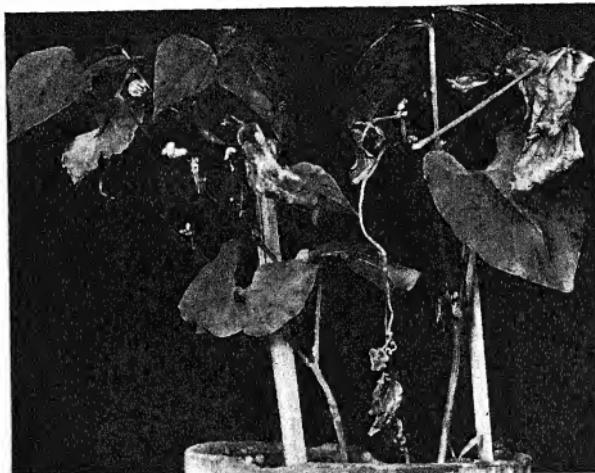


FIG. 4.—Bean plants 21 days after inoculation with *B. phaseoli* no. 2; top completely dead in one plant.

sojense are similar but distinct from *B. flaccumfaciens* in their action upon starch agar, gelatin, and sugars.

Table V shows that the antiserum from *Bact. phaseoli sojense S* is highly specific for its homologous organism, and will differentiate this from the other two organisms. Antiserum prepared from *Bact. phaseoli sojense R* gave the same results as that from the *S* strain; however, no agglutination tests could be run against the *R* strain, since this is spontaneously agglutinated in both distilled water and in salt solution. Since the antisera from both organisms act alike, it is probable that they are chemically the same, and differ only in

their physical properties. Serologically they both differ from *Bact. phaseoli* and *Bact. flaccumfaciens*. Table VI shows that none of the organisms was agglutinated by normal rabbit serum.

B. PRECIPITIN TEST

GRIFFITH (8), and JACOBSON and FALK (14, 15) have shown that when *S* and *R* strains of pneumococci are grown in broth, the former produces in 4–16 hours a specific soluble substance which when

TABLE VII
PRECIPITIN TEST USING ANTISERUM FROM *B. PHASEOLI SOJENSE SMOOTH*, AGAINST FILTERED BROTHS FROM SMOOTH AND ROUGH STRAINS OF *B. PHASEOLI SOJENSE R*

AMOUNT OF SERUM (CC.)	SOURCE OF BROTH	AMOUNT OF BROTH (CC.)	RESULTS
0.5	Smooth	0.5	++++
0.25	Smooth	0.75	+++
0.125	Smooth	0.875	+
0.5	Rough	0.5	++++
0.25	Rough	0.75	++++
0.125	Rough	0.875	+
0.5	(Sterile control)	0.5	—
0.25	(Sterile control)	0.75	—
0.125	(Sterile control)	0.875	—

mixed with homologous antiserum will give the precipitin reaction. The *R* strain gives no precipitation early, however, but after 24–48 hours will give a reaction with its antiserum.

Since it was found possible to differentiate *S* and *R* strains of pneumococci in this manner, it was decided to apply this technique to *Bact. phaseoli sojense S* and *R* strains, to see whether it could also be used to differentiate these strains. Table VII gives the protocol of the experiment. Standard beef extract-peptone-dextrose broth was used. The organisms were allowed to grow in separate flasks for 8 days, after which the culture media were passed through Berkefeld filters to obtain a clear solution free from bacteria. When antiserum prepared from either *S* or *R* strains was added to the filtrates in suitable quantities a precipitation occurred in each case, showing

that the test was non-specific for the strains. No precipitation was obtained when sterilized plain broth was used. The test was repeated later but the organisms were allowed to grow only 6 days. No precipitation was obtained. The conclusions are that (1) the first precipitation obtained was probably due to autolytic products of the bacteria

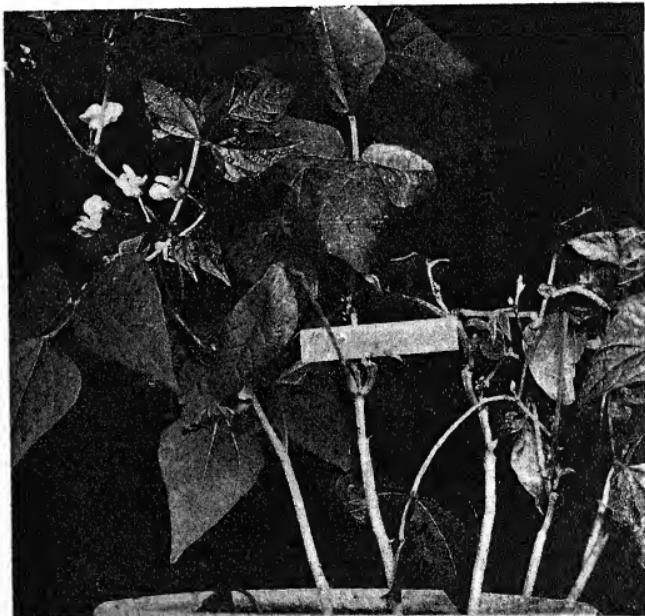


FIG. 5.—Keeney beans 19 days after being inoculated with *B. phaseoli* no. 2

and not to any soluble substance produced; (2) the *S* and *R* strains cannot be differentiated by the precipitin test when filtrates of broth cultures are used.

IV. Virulence

BACT. FLACCUMFACIENS AND BACT. PHASEOLI

In studying virulence of the pathogens under consideration, the following varieties of beans were used: Challenge Dwarf Black Wax, Keeney's Rustless Golden Wax, and Manchu soy bean. *Bact.*

flaccumfaciens no. 7 and no. 8, *Bact. phaseoli* no. 1 and no. 2, and *Bact. phaseoli sojense S* and *R* respectively were inoculated into these varieties.

In all experiments on virulence the plants were inoculated by pricking with a needle, by spraying, or by rubbing the leaves and



FIG. 6.—Challenge beans 19 days after being inoculated with *B. phaseoli* no. 2; lesions much more severe than in fig. 5.

stems. After the plants were inoculated they were set in a moist chamber for 48 hours, and then removed to the greenhouse where the temperature ranged from 75°–112° F. In every case the organisms were much more virulent at the higher temperatures.

Bact. flaccumfaciens no. 7 when pricked or rubbed into the leaves or stems of the Challenge or Keeney bean produced only slight infec-

tion, only two or three leaves showing wilting or lesions at any one time (fig. 1). This strain of the organism was never reisolated from the plant. Culturally and serologically it was the same as no. 8. *Bact. flaccumfaciens* no. 8 when inoculated into the same varieties by the same method used for no. 7 produced wilting in 9 days, signs of which appeared as early as the fourth day. The plants soon ceased to grow, and at the end of 21 days were almost dead (fig. 2). The non-inoculated plant in the same pot remained healthy, thus confirming statements in the literature that this organism gains entrance only through wounds. No infection was ever produced in the soy bean by either no. 7 or no. 8. Strain no. 8 was reisolated from diseased plants and upon further inoculations again proved pathogenic.



FIG. 7.—Characteristic lesions on soy bean leaf produced by *B. phaseoli sojense*; inoculations made by needle pricks.

no. 2 in every experiment performed (figs. 3, 4). When the plants were inoculated by injecting suspensions of the organisms into the stem no difference in virulence was noted in no. 1 and no. 2. Production of the disease by stem inoculation showed that the organism can invade the vascular system when wounds are present. Yellow exudations of bacteria were formed at the point of inoculation. It was also found that in every instance the Keeney bean was more resistant to both *Bact. phaseoli* and *Bact. flaccumfaciens* than was the Challenge bean (figs. 5, 6). Both no. 1 and no. 2 were reisolated and proved pathogenic upon reinoculation.

BACT. PHASEOLI SOJENSE S AND R

When suspensions of the *S* and *R* strains were inoculated into the leaves of soy beans by spraying or rubbing no difference in the

pathogenicity of these strains was observed; however, when the leaves were inoculated by needle pricks a difference in percentage of infection and in size of lesions was noted. In making the needle prick inoculations, the needle was dipped into a suspension of the organism, and five punctures made in the leaf, after which the needle was again placed in the inoculum and five more punctures made. This operation was continued until 150 punctures were made with each of the strains. Following inoculation the plants were kept in a

TABLE VIII

PERCENTAGE OF INFECTION ON DIFFERENT DAYS RESULTING
FROM 150 PUNCTURES WITH SUSPENSIONS OF BACT.
PHASEOLI SOJENSE SMOOTH AND ROUGH

EXPERIMENT	PERCENTAGE OF INFECTION			REMARKS
	4 days	5 days	6 days	
1. Smooth.....	56	80		
Rough.....	26	74		
2. Smooth.....	64	91.8		
Rough.....	30	88.0		
3. Smooth.....	71		
Rough.....	66		
4. Smooth.....	39		
Rough.....	7		In 8-10 days there was practically 100 per cent infection from both smooth and rough; lesions from smooth organisms were always larger with larger and much more definite yellow borders than from rough
5. Smooth.....	90		
Rough.....	57		
6. Smooth.....	75		
Rough.....	52		

moist chamber for 48 hours, and then removed to the greenhouse where daily observations were made. In each of the six experiments infection took place more rapidly with the *S* than with the *R* strain (table VIII).

The lesions produced by the *S* strain always appeared larger than those produced by the *R* strain. In order to determine that they really were larger they were accurately measured. The leaves were inoculated by needle pricks (fig. 7), and when the lesions had developed, after 5-8 days, the leaves were removed from the plants. They were next placed between two large pieces of glass which were

bound together by adhesive tape, so that measurements could be made under low power of the microscope by use of an ocular microm-

TABLE IX
SIZE OF LESIONS PRODUCED ON SOY BEANS BY BACT. PHASEOLI
SOJENSE SMOOTH AND ROUGH

Di- a- me- ter*	EXPERIMENT NO. 1 INOCULATED AUGUST 20; MEASURED AUGUST 28, 1926				EXPERIMENT NO. 2 INOCULATED AUGUST 22; MEASURED AUGUST 28, 1926				EXPERIMENT NO. 3 INOCULATED AUGUST 25; MEASURED AUGUST 30, 1926				
	Smooth		Rough		Smooth		Rough		Smooth		Rough		
	Dia- meter	F†	Dia- meter	F	Dia- meter	F	Dia- meter	F	Dia- meter	F	Dia- meter	F	
18	1	14	1	12	1	12	1	12	2	10	2		
21	2	18	3	19	1	14	1	16	5	11	2		
22	3	20	4	20	4	15	3	16	5	12	5		
23	1	21	3	21	1	16	1	17	5	13	4		
24	1	22	3	22	1	17	1	18	5	14	9		
25	2	23	3	24	2	18	1	19	5	15	9		
26	1	24	1	25	1	19	2	20	10	16	11		
28	1	25	3	27	2	20	3	21	6	17	7		
29	2	26	1	28	3	21	1	22	10	18	8		
30	2	27	1	29	1	22	1	23	10	19	4		
31	1	28	2	30	6	23	3	24	7	20	20		
32	1	29	1	31	1	24	1	25	5	21	8		
33	1	30	2	32	3	25	7	26	6	22	6		
34	5	31	1	33	5	26	1	27	6	23	8		
35	2	32	1	34	1	27	3	28	5	24	2		
36	2	33	1	35	4	28	5	29	3	25	4		
37	1	34	2	37	2	30	6	30	11	27	3		
38	2	35	12	38	2	31	1	32	5	28	4		
40	7	36	1	40	2	32	2	33	2	30	4		
41	1	37	1	42	1	33	1	34	7	32	2		
42	2	38	2	43	1	36	1	36	6	33	2		
43	1	39	3	45	3	40	3	37	2	34	1		
45	5	40	3	50	2	50	1	40	5	36	2		
48	3	41	1	44	2	37	1		
50	5	42	1	45	7	38	1		
52	3	43	3	50	6	40	1		
53	1	44	1	52	1	41	1		
55	3	45	5	55	5		
58	2	46	2	57	1		
65	4	52	1	60	2		
Total.....	2734	68	2238	69	2500	50	2292	50	4654	161	2427	161	
Average size...	40.2 ± 0.49		32.43 ± 0.36		31.8 ± 0.30		25.84 ± 0.44		28.92 ± 0.30		20.4 ± 0.2		
Average size mm.....	1.61 ± 0.02		1.28 ± 0.014		1.26 ± 0.016		1.03 ± 0.018		1.16 ± 0.012		0.81 ± 0.008		

* "Diameter" is for diameter of lesions on soy bean leaves measured in ocular spaces. One ocular space equals 0.04 mm.

† F is for frequency of occurrence of lesions as indicated by diameter in ocular spaces to the left.

eter and direct illumination. The data obtained from these measurements are recorded in tables IX and X. They show that for all six of the experiments the average size of the lesions produced by the

S strain is about 1.28 times as large as that produced by the *R* strain (table XI). The *S* strain used in experiment 6 recorded in table X was reisolated from a soy bean that had been inoculated with an *R* strain. It is significant that the average size of the lesions in this experiment is 1.29 times that of the lesions caused by the *R* strain.

TABLE X
SIZE OF LESIONS PRODUCED ON SOY BEANS BY BACT. PHASEOLI
SOJENSE SMOOTH AND ROUGH

EXPERIMENT NO. 4 INOCULATED SEPTEMBER 2; MEASURED SEPTEMBER 8, 1926				EXPERIMENT NO. 5 INOCULATED SEPTEMBER 6; MEASURED SEPTEMBER 12, 1926				EXPERIMENT NO. 6 INOCULATED SEPTEMBER 7; MEASURED SEPTEMBER 12, 1926				
Smooth		Rough		Smooth		Rough		Smooth		Rough		
Diameter*	F†	Diameter	F	Diameter	F	Diameter	F	Diameter	F	Diameter	F	
12	2	10	5	12	1	9	1	12	4	9	3	
13	2	11	8	13	4	10	4	13	7	10	6	
14	2	12	8	14	7	11	9	14	5	11	13	
15	8	13	7	15	19	12	16	15	14	12	10	
16	8	14	12	16	10	13	16	16	14	13	20	
17	13	15	15	17	8	14	8	17	11	14	15	
18	16	16	4	18	13	15	12	18	14	15	21	
19	12	17	8	19	5	16	13	19	9	16	3	
20	7	18	9	20	12	17	6	20	13	17	15	
21	5	19	3	21	1	18	3	21	6	18	5	
22	3	20	4	22	2	19	1	22	4	20	2	
24	5	21	1	23	2	21	1	23	5	21	1	
25	2	22	2	24	3	24	2	22	1	
27	1	23	2	25	2	25	1	23	1	
29	1	25	1	30	1	26	4	24	1	
30	2	27	1	
32	1	30	2	
Total.....	1692	90	1353	89	1575	90	1244	90	2106	116	1712	120
Average size... (mm.).....	18.8 ± 0.14		15.18 ± 0.12		17.5 ± 0.12		13.82 ± 0.084		18.15 ± 0.12		14.26 ± 0.09	
	0.751 ± 0.0056		0.608 ± 0.0048		0.696 ± 0.0048		0.552 ± 0.0032		0.724 ± 0.0048		0.5700 ± 0.0036	

* "Diameter" is for diameter of lesions on soy bean leaves measured in ocular spaces. One ocular space equals 0.04 mm.

† F is for the frequency of occurrence of lesions as indicated by diameter in ocular spaces to the left.

The probable error (23) was calculated for each set of figures of the different experiments. It is seen that the figures obtained are too small to affect the validity of the data.

The conclusions drawn from the experiments on virulence are as follows: (1) The *S* strain is more virulent than the *R* strain; it produces infection earlier and also produces larger lesions. (2) The *R*

strain can be changed to the *S* strain by passage through the soy bean plant, the *S* strain so obtained being fully as virulent as the regular *S* strain. (3) The results in these experiments demonstrate concordance between phenomena in plants with those previously reported in animal bacteriology (1, 14, 15, 16).

V. Acid agglutination

The phenomenon of acid agglutination has been studied by NORTHROP and coworkers (20, 21), DEKRUIF (3, 4), and by EGGERTH and BELLOWS (7). The last two workers found that some

TABLE XI
SUMMARY OF DATA ON PATHOGENICITY OF BACT. PHASEOLI
SOJENSE SMOOTH AND ROUGH, ON SOY BEANS AS DETER-
MINED BY SIZE OF LESIONS

EXPERI- MENT NO.	AGE WHEN MEASURED (DAYS)	SMOOTH		ROUGH		RATIO SMOOTH ROUGH
		Number measured	Average size of lesions (mm.)	Number measured	Average size of lesions (mm.)	
1.....	8	68	1.61 ± 0.02	69	1.28 ± 0.014	1.25
2.....	6	50	1.26 ± 0.016	50	1.03 ± 0.018	1.22
3.....	5	101	1.16 ± 0.012	101	0.81 ± 0.008	1.43
4.....	6	90	0.751 ± 0.006	89	0.668 ± 0.005	1.23
5.....	6	90	0.696 ± 0.005	90	0.552 ± 0.003	1.26
6.....	5	116	0.724 ± 0.005	120	0.57 ± 0.004	1.29
Total measured....		575	579
General average....			1.033		0.808	1.28

strains of *Bact. coli* would agglutinate in dilute acids without salts at reactions ranging from P_H 1.6 to 3.0.

When the bacterial suspensions were being made for injecting the rabbits in the serological tests, it was noticed that suspensions of the rough strain of *Bact. phaseoli sojense* agglutinated in 0.85 per cent salt solution. DOCHEZ, AVERY, and LANCEFIELD (5, 6) found that if streptococci, which had a tendency to clump, were washed in distilled water, these would remain in suspension. In the light of their findings suspensions were again made of each of the organisms under study, and these suspensions were washed nine times in distilled water. All the organisms remained in suspension except the *R* strain, which precipitated just as rapidly after the ninth washing

as after the first. Next, suspensions of all the organisms were prepared to determine the range of P_H at which they would agglutinate, and to see whether they could be differentiated on this basis.

The isoelectric point for *Bact. flaccumfaciens* no. 7 and no. 8 was found to be between P_H 1.2 and 3.0 (table XII and fig. 8a). Obviously they cannot be differentiated on this basis. The isoelectric point of *Bact. phaseoli* no. 1 and no. 2 (table XIII and fig. 8b) is almost

TABLE XII
INFLUENCE OF P_H ON AGGLUTINATION OF WASHED BACT.
FLACCUMFACIENS IN DISTILLED WATER

P_H	BACT. FLACCUMFACIENS NO. 7			BACT. FLACCUMFACIENS NO. 8		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
5.5.....	—	—	—	—	—	—
4.5.....	—	—	—	—	—	—
3.5.....	—	—	—	—	—	—
3.0.....	—	+	—	—	+	—
2.8.....	+	+	+++	—
2.7.....	+++	++
2.6.....	+	—	+	—
2.5.....	+++	—	—
2.4.....	+++	—
2.3.....	—	+++	—
2.2.....	+++	++	—	—
2.0.....	+++	+	—	+++	+++++c	—
1.8.....	++	+	—	++	+++++c	—
1.6.....	+	+	—	+	+++++c	—
1.4.....	+	—	—	+	+++++c	—
1.2.....	—	—	—	+	+++	—
1.2—	—	—	—	—	—	—
**.....	P_H 6.4—	P_H 6.8—	P_H 6.5—	P_H 6.4—	P_H 6.8—	P_H 6.2—

** Control suspension with no acid added.

c, Cloudy, almost complete agglutination.

identical, and does not differ materially from that of *Bact. flaccumfaciens*.

Bact. phaseoli sojense S was found to have a much narrower range of agglutination than strain *R*; in fact strain *R* agglutinated through practically the entire range of P_H 2.0 to 9.4, little or no agglutination occurring from P_H 5.5 to 5.8 (table XIV and fig. 8c). The spontaneous agglutination of strain *R* will easily differentiate it from all the other organisms studied.

It has been found by JACOBSON and FALK (14) that certain animal pathogens show a correlation between acid agglutination and the

CHART A

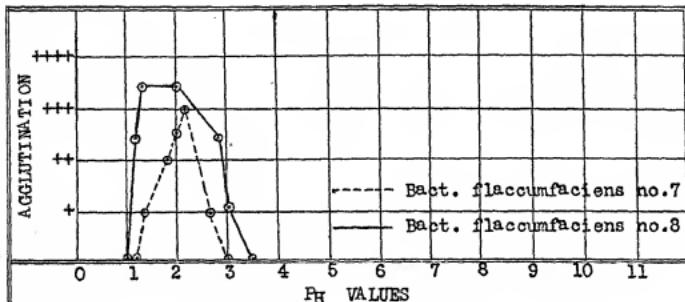


CHART B

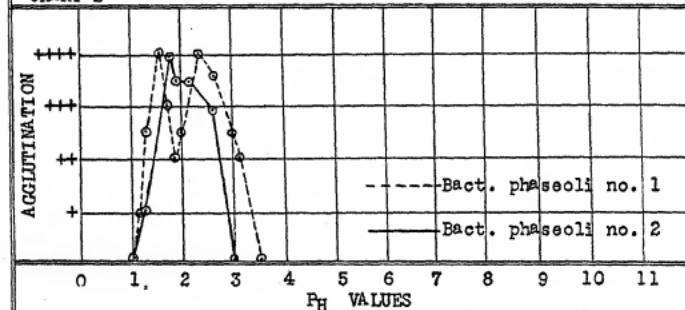


CHART C

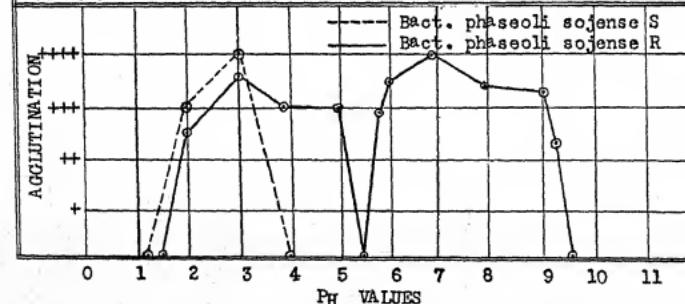


FIG. 8.—Acid agglutination of: a, *B. flaccumfaciens*; b, *B. phaseoli*, and c, *B. phaseoli sojense*, at various P_H ranges.

TABLE XIII
INFLUENCE OF P_h ON AGGLUTINATION OF WASHED BACT.
PHASEOLI NO. 1 AND NO. 2 IN DISTILLED WATER

P_h	No. 1							No. 2
	No. 1	Exp. 2	Exp. 3†	Exp. 4	Exp. 5	Exp. 6	Exp. 7	
6.8*	—	—	—	—	—	—	—	—
6.6*	—	—	—	—	—	—	—	—
6.2*	—	—	—	—	—	—	—	—
3.1.	+	—	—	—	—	—	++	—
3.0.	++	—	+	+	+	—	+++	—
2.8.	+++	—	—	+	—	—	—	—
2.6.	—	++	+++++c	—	+++++c	+++	+++++c	+++
2.4.	+++	+	+++++c	+++++c	++	++++	++++	—
2.2.	+	—	—	—	—	—	—	+++++c
2.0.	+	—	+++	+++++c	+	++++	+++	—
1.8.	++	—	++	++	+	++++	++	+++++c
1.7.	+++	—	++	++	+	++	+++	++++
1.6.	—	—	++	++	+	—	+++	—
1.4.	+	—	—	+	—	—	+++	—
1.2.	—	—	—	—	—	—	+	—

* P_h of original washed suspension before acid was added.

† Organism reisolated from the bean.

c, Cloudy, almost complete agglutination.

TABLE XIV
INFLUENCE OF P_h ON AGGLUTINATION AND ELECTROPHORETIC POTENTIALS
OF WASHED BACT. PHASEOLI SOJENSE SMOOTH
AND ROUGH, IN DISTILLED WATER

WASHED						ANOTHER WASHED SUSPENSION OF ROUGH PREPARED FROM SAME ORIGINAL, 24 HOURS LATER			
Smooth			Rough			P_h	Agg.	P_h	Agg.
P_h	Agg.	P.D. $\mu/sec.$ †	P_h	Agg.	P.D. $\mu/sec.$	P_h	Agg.	P_h	Agg.
10.	—	—	10	Dissolved	—	9.6	+	6.0	+
9.	—	—	9	Dissolved	—	9.4	+++	5.5	—
8.	—	—	8	+++++c	—113.5	9.2	+++++c	5.0	+++
7.0.	—	—	7.1*	+++++c	—50.0	9.0	+++++c	4.6	+++
6.6*	—	-52.2	6.0*	+++++c	-07.0	8.9	+++++c	3.1	+++
6.0.	—	—	5.8	+++	-60.7	8.2	+++	3.0	+++
5.0.	—	—	4.8	+++	-38.6	7.4	+++	2.8	+++
4.0.	—	—	3.9	+++	-30.2	7.3	+++	2.6	+++
3.0.	+++	-4.3	3.0	+++++c	14.6	7.2	+++	2.2	+++
2.0.	+++	-5.3	2.0	+++	+22.3	7.1	+++	2.0	+++
1.2.	—	0	1.2	—	—	7.0*	+++	1.9	—
						6.8	+++	1.8	+
						6.6	+++	1.6	—

*Control; nothing added.

† P.D. determinations made by Dr. I. S. FALK.

c, Cloudy, almost complete agglutination.

electrophoretic potential difference between the organism and the suspending menstruum. In preliminary experiments a similar correlation (table XIV) between the *S* and *R* strains of *Bact. phaseoli sojense* has been found in the acid but not in the alkaline range. Additional work is in progress, and the protocol and discussion of this phase of the work will be published later.³

The conclusions drawn here are: (1) The isoelectric point of each of these organisms is between P_H 1.2 and 3.0. (2) The *R* strain of *Bact. phaseoli sojense* can be differentiated, on the basis of acid agglutination, from the *S* strain and from the other organisms as well. (3) Roughness and lesser virulence are correlated with greater agglutinability, and smoothness with greater virulence and less agglutinability. (4) For the *R* and *S* strains there appears to be a direct correlation between the P.D. and agglutination in the acid zone, with no agglutination when the P.D. is high and with complete agglutination when the P.D. is low.

Summary and conclusions

1. A morphological, physiological, and serological study, together with virulence and acid agglutination studies have been made of *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*.

2. Two strains, no. 7 and no. 8, were isolated from a pure culture of *Bact. flaccumfaciens*. Strain no. 7 was only slightly pathogenic for beans, while no. 8 was very pathogenic. These strains also differed morphologically in the kind of colony produced, and in appearance on agar slants and plates; otherwise they were identical both as to their physiological and serological reactions, as well as to acid agglutination.

3. Two strains, no. 1 and no. 2, were isolated from a pure culture of *Bact. phaseoli*. These differed slightly as to morphological and cultural characteristics and in virulence; but were indistinguishable serologically and by the acid agglutination test.

4. A smooth (*S*) and a rough (*R*) strain of *Bact. phaseoli sojense* were isolated from a pure culture of *Bact. phaseoli sojense* which macroscopically appeared smooth.

³ FALK, I. S., SHARP, C. G., and LINE, G. K. K., Relation between P_H , agglutination, and P.D., with *Bact. phaseoli sojense*. Proc. Soc. Exp. Biol. and Med. 1927. (In press).

5. The *S* and *R* strains were identical physiologically and serologically, but differed morphologically, in virulence, and in range of acid agglutination.

6. Colonies of the *S* strain in potato dextrose agar were always smooth, flat, and spreading, while colonies of the *R* strain were always rough and round, growing vertically instead of spreading, thereby forming umbonate colonies. On thickly sown plates umbilicate and uneven forms were noted.

7. The *S* strain remained constant throughout the investigation, neither changing in culture media nor during passage through soy bean plants.

8. The *R* strain remained constant in culture media, but when this strain was inoculated into soy bean plants and reisolations made, both *S* and *R* colonies appeared.

9. The *S* strain was more virulent for soy bean, both as to time of appearance and size of lesions. The *S* strain, reisolated from lesions produced by inoculation of the *R* strain into the plant, was fully as virulent as that recovered from lesions following inoculation with an *S* strain, and also was more virulent than the *R* strain from which it came.

10. The range of acid agglutination for the *S* strain was between P_H 1.2 and 4.0, while the *R* strain agglutinated practically all the way from P_H 2.0 to 9.4, except from P_H 5.5 to 5.8, where little or no agglutination occurs.

11. Nine washings in distilled water by centrifuging failed to prevent the *R* strain from being spontaneously agglutinated in distilled water.

12. Greater virulence of the *S* strain seems to be correlated with motility and lesser agglutinability, and lesser virulence of the *R* strain with low motility or non-motility and with greater agglutinability.

13. In the acid zone there also appears to be a correlation between low P.D. and high agglutination, and between high P.D. and low agglutination, but not in the alkaline.

14. *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* all differ serologically, and can be differentiated by the use of the agglutination test. *Bact. flaccumfaciens* serologically stands apart

from *Bact. phaseoli* and *Bact. phaseoli sojense*, which are quite closely related. This is in harmony with the cultural findings.

15. Culturally *Bact. phaseoli* and *Bact. phaseoli sojense* are practically identical, except for slight differences in final P_H after 30 days, and in difference in character of growth in lactose and levulose broth. *Bact. flaccumfaciens* can easily be distinguished from these in all culture media used.

16. The isoelectric point for *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* is between P_H 1.2 and 3.0 for all three organisms. The isoelectric point for the *S* and *R* strains is approximately the same in the acid zone.

17. All of these organisms are more virulent to plants when the temperature is high.

The writer wishes to express his great appreciation to Dr. GEO. K. K. LINK and Dr. I. S. FALK, for their many helpful suggestions during the process of this investigation

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EXPLANATION OF PLATE VII

FIG. 9.—Colonies of *B. phaseoli* no. 1, 8 days old, showing concentric rings and thin hyaline borders.

FIG. 10.—Colonies of *B. phaseoli* no. 1, 15 days old, showing increased number of concentric rings and thin border.

FIG. 11.—Colony of *B. phaseoli* no. 2, 8 days old, with no concentric rings or hyaline borders.

FIG. 12.—Smooth colony of *B. phaseoli sojense*, 4 days old, obtained by planting culture with needle.

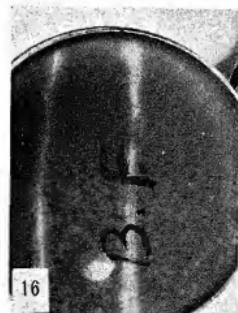
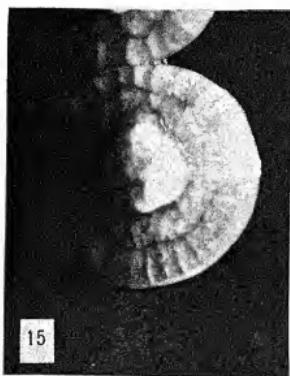
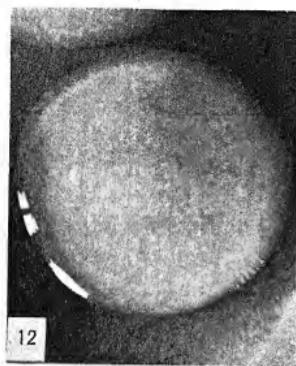
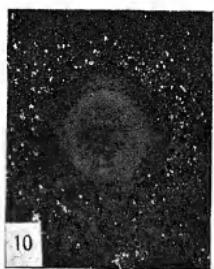
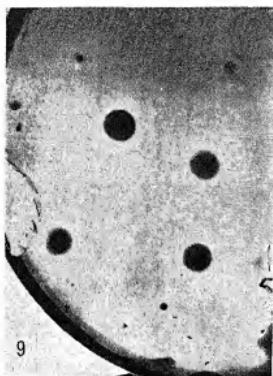
FIG. 13.—Rough colonies of *B. phaseoli sojense*, 8 days old, obtained by agar shake cultures and pouring plates.

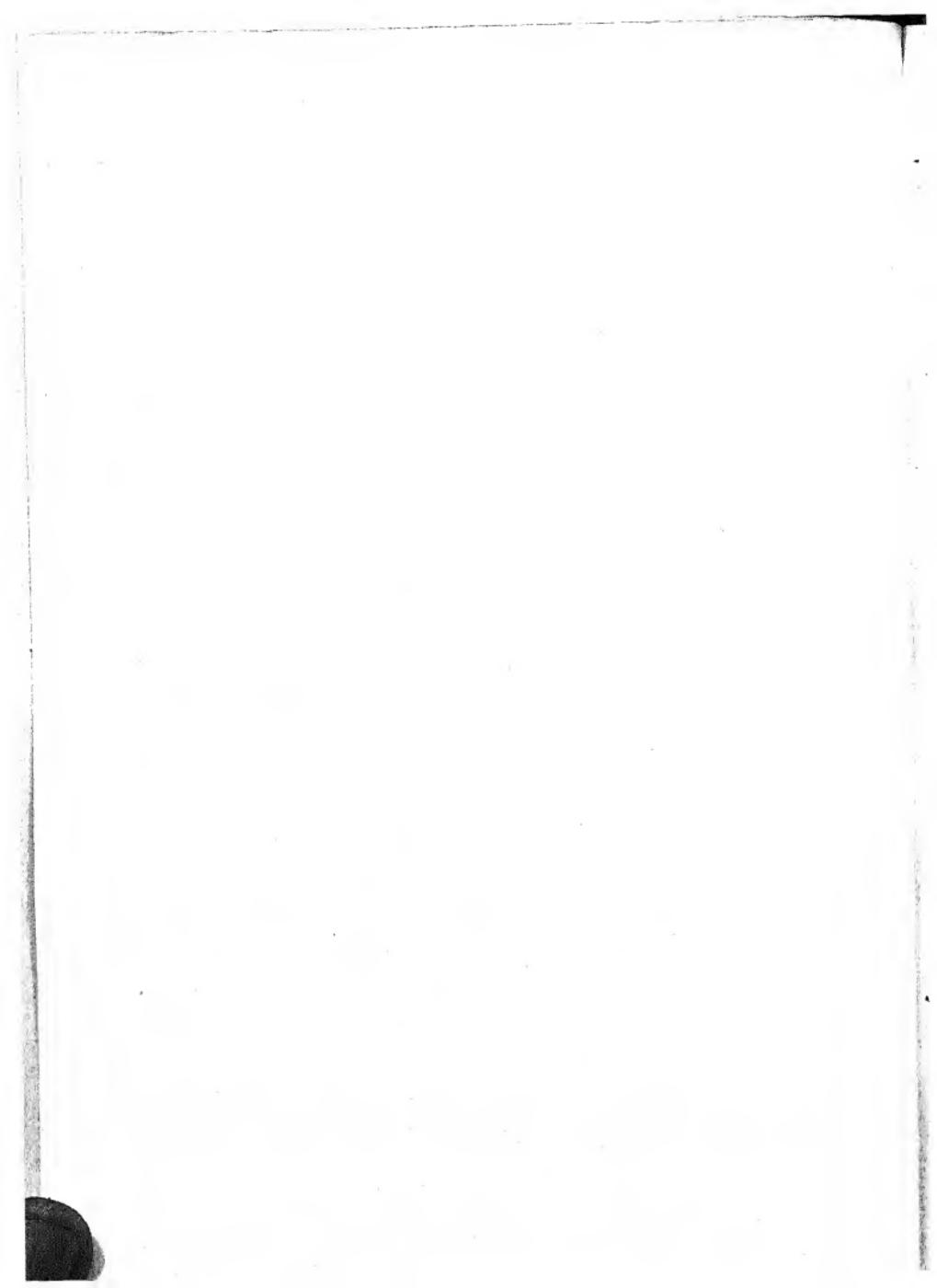
FIG. 14.—Rough colony of *B. phaseoli sojense*, 4 days old, obtained by planting culture with needle.

FIG. 15.—Rough colony of *B. phascoli sojense*, 8 days old, obtained by making agar shake cultures and pouring plates; note wrinkles and umbonate form.

FIG. 16.—Hydrolysis of starch in 3 days by *B. flaccumfaciens*.

FIG. 17.—Hydrolysis of starch in 3 days by *B. phaseoli*.





CORRELATION OF HOST AND SEROLOGICAL SPECIFICITY OF BACTERIUM CAMPESTRE, BACT. FLACCUM-FACIENS, BACT. PHASEOLI, AND BACT. PHASEOLI SOJENSE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 365

G. K. K. LINK AND C. G. SHARP

Introduction

This paper and the accompanying one by SHARP have arisen out of the project of the pathology division of the Hull Botanical Laboratory for a study of the phenomena of resistance or immunity-susceptibility as seen in the field of phytopathology.¹ The phenomena of resistance or immunity-susceptibility in plants, with their vast array of many types and all degrees of biological specificity, constitute one of the most interesting and intriguing problems of theoretical and applied phytopathology. There is no need of cataloging and discussing the recorded illustrations of these phenomena. A partial enumeration has recently been made by WALKER (39).

Serological investigations have played an important rôle in the study of virulence and of the resistance-susceptibility phenomena in the fields of animal immunology and pathology. Animal immunologists have used plant materials to study the properties of plant protein as antigens, notably WELLS and OSBORNE (41), and many biochemists have studied the chemistry of these proteins following OSBORNE (27). In spite of the interesting and very suggestive findings by bacteriologists, zoologists, and biochemists, plant pathologists have practically ignored serological methods in attacking their problems.

The work here reported is the result of an application of serological methods to a study of the biological specificity shown by cer-

¹ In the work reported in these papers we had the active cooperation of the department of hygiene and bacteriology of the University of Chicago, which supplied and kept the animals used. We are indebted to Dr. I. S. FALK and Dr. W. H. TALLAFERRO of that department for criticism of the data presented.

tain yellow schizomycetes which are pathogenic to plants. *Bacterium campestre*, *Bact. citri*, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. juglandis*, *Bact. pruni*, and *Bact. translucens* are all yellow schizomycetes which are not readily distinguishable in culture, but which show marked biological specificity in their host relations. Thus *Bact. campestre* causes black rot of cabbage, *Bact. citri* canker of citrus, *Bact. malvacearum* angular leaf spot of cotton, *Bact. juglandis* walnut blight, *Bact. phaseoli* bean blight, *Bact. pruni* black spot and canker of peach and plum, and *Bact. translucens* black chaff of wheat.

SMITH (34), in discussing the pathogene of angular leaf spot of cotton, *Bact. malvacearum*, states as follows:

In various ways this organism resembles *Bacterium campestre*, *Bacterium phaseoli*, and *Bacterium citri* but I did not succeed in cross inoculating it to cabbages, to beans, or to oranges. Further comparison should be made not only with *Bacterium campestre*, *Bacterium citri*, and *Bacterium phaseoli*, but also with *Bacterium pruni*, *Bacterium juglandis*, and *Bacterium translucens*, all of which are closely related. Indeed, some of the names are perhaps synonymous, but this can be settled only by many cross inoculations and much further study.

In his discussion of *Bact. phaseoli*, the pathogene of blight of beans, he writes:

On a variety of culture media *Bacterium phaseoli* is closely like *Bacterium campestre*, but the two organisms are not identical, as shown by the failure of repeated cross inoculations (cabbage bacterium on beans and bean bacterium on cabbages), but our present means of separating the two forms culturally is insufficient. It is also culturally much like *Bacterium citri*, but with a virulent strain I failed to obtain any scabs in *Citrus decumana* (about 60 young seedlings). The student, therefore, who has opportunity might direct his attention to comparative studies of the yellow organisms of this group in the hope of finding additional differences by the use of new media. But in any event, *Bacterium phaseoli* belongs with *Bacterium campestre*, *Bacterium hyacinthi*, *Bacterium vascularum*, *Bacterium pruni*, *Bacterium malvacearum* (no. X), *Bacterium citri*, and *Bacterium translucens* in a closely related kinship.

It is obvious from these quotations that there is apparent biological specificity in the pathogenes discussed, so far as host relations are concerned, and that in the main this has been the reason for considering these organisms as distinct species. The same problem in a more refined state exists, so far as various bean pathogenes are concerned, where we have *Bact. phaseoli*, the pathogene of bean blight, *Bact. flaccumfaciens*, the cause of bean wilt, and *Bact. phaseoli* so-

jense, the pathogene of bacterial pustule of the soy bean. This situation has been investigated by SHARP in the accompanying paper.

It seemed worth while to determine whether serological technique, specifically the agglutination test, could be used to differentiate these microorganisms, and possibly in addition to tell something about their relationship. A positive result would give another and needed proof that these organisms are deserving of species rank. More important theoretically, however, was the consideration that if we should find serological specificity correlated positively with biological specificity, so far as host relations are concerned, there would be some promise that serological technique might become a tool for the study of resistance-susceptibility and virulence phenomena in phytopathology. We were thus carried into the field of sero-diagnosis, which has been used extensively by bacteriologists and animal immunologists, but which botanists with a few exceptions have left practically untouched. A perusal of the literature on sero-diagnostic methods applied to microorganisms, in the main, promised that we might be successful; but there also were indications that we might fail.

GRUBER and DURHAM (12), while studying the bactericidal reaction with colon bacilli and cholera spirilla, noticed that homogeneous suspensions of these microorganisms were agglutinated or clumped by their own or homologous antisera; but not by heterologous sera. They recognized the specificity of the reaction, and proposed that the method could be used for bacterial differentiation and species determination. This method (the agglutination or agglutinin test) has become a very important tool of almost universal application in the hands of the immunologist and bacteriologist, and is used most frequently for rapid identification of colonies of doubtful typhoid or dysentery. By this method the dysentery bacillus was discovered by SHIGA. The Gruber-Widal test, which is the converse of the preceding method, is used generally for the early diagnosis of typhoid fever. The agglutination test has also been used to differentiate and classify microorganisms which are impossible to separate by means of cultural, inoculation, and microscopic tests. Thus, BUTTERFIELD and NEILL (5) have demonstrated that all agglutinable strains of meningococci may be classified under four types.

JONES (15) showed that sixteen strains of *Bacillus bovisepicus* can on serological and cultural grounds be put into three groups. On the other hand, Miss EVANS (7) found that serologically she could not differentiate the strain of *Brucella melitensis*, which is associated with malta fever in man, and the strain associated with abortion in cattle.

The discovery of GRUBER and DURHAM led to experimentation which demonstrated that agglutination or clumping could be brought about, not only by antisera produced by injection into suitable animals of whole bacteria as GRUBER and DURHAM had done, but also by injection of dissolved extracts of bacteria or of filtrates of the old culture media. Following this line of investigation, KRAUS (18) found that clear filtrates of the broth cultures of *Bacillus pestis* and the cholera spirillum, when mixed with their respective antisera, gave at first turbidity and finally a precipitate. He found that the reaction was specific, and pointed out the practical diagnostic possibilities of the discovery. He named this the "precipitin reaction," having thus discovered the precipitation or precipitin test which also has become extremely important in serological technique.

This method has been used in the diagnosis of glanders by WLA-DIMIROFF, and is an important adjunct to other methods in determining the biological relationship of microorganisms. Its greatest practical use, however, is outside the field of microorganisms, because it was soon found that specific antisera are produced whenever animals are injected with any kind of foreign proteins, bacterial, plant, or animal. Practically, the precipitin test is used extensively in forensic medicine and in determining the adulteration of meat products and of meals (flour). In theoretical biology it has been used to determine phylogenetic relationship of animals, especially by NUTTALL (26), and of plants most extensively by MEZ and his co-workers (24), and by GOHLKE (11).

The relative infrequency of bacterial diseases of plants in Europe, as well as long delayed recognition by German bacteriologists of the rôle of schizomycetes as plant pathogens, probably were factors responsible for the fact that serological technique was not at once applied to these organisms by bacteriologists and botanists when the wave of serological testing swept through the ranks of biologists and the medical profession in Europe. As a result, the first attempts to

use the new technique in the botanical field outside of bacteriology were made in connection with experiments to determine whether the precipitation test could be employed to detect adulteration in flours and meals. Following the earlier work of KOWARSKI (17) and BERTARELLI (3), which opened the way but which was not conclusive, a considerable number of investigators have touched this field, especially MAGNUS (22,23), MAGNUS and FRIEDENTHAL (21), and RELANDER (29). WENDELSTADT and FELLMER (40) used the precipitin as well as the complement fixation test. SAULI (31) employed the conglutination test. GASIS (9) and BECKER (2) introduced more refined quantitative methods, and as a result the latter found that he could detect an adulteration of 0.125 per cent (0.000001 gm.) of *Argostemma Gigathi* in wheat flour. He also applied the method to detection of foreign seeds in seed stocks. More recently these methods have been used in connection with genetic studies in separating and identifying strains and varieties of agricultural plants, especially by ZADE (43) and ARZT (1).

SCHÜTZE (32) in 1903 attempted an application of serological technique to a mycological problem. He endeavored to differentiate races or varieties of yeasts, but without success. CITRON (6) tried to apply the methods to differentiate the *Favus* from the *Trichophyton* fungi, but without success. The first encouraging results in the application of serological technique to problems of this type were obtained by MAGNUS and FRIEDENTHAL (20). These investigators endeavored to determine whether serological test methods would support the deductions of morphological systematists as to relationship of fungi based on comparative morphology. They used extracts of *Saccharomyces cerevisiae*, *Tuber brumale*, and *Agaricus campestris* as antigens. Their findings corroborated the current opinion of the relationship of these three fungi.

ROSENBLATT-LICHENSTEIN (30) in 1912 made serological tests using, in pure culture, six strains of algae, whose identity, however, was not determined. In 1916 LIESKE (19) applied serological methods to a study of unicellular green algae. He made use of all the methods, agglutination, precipitation, and complement fixation, obtaining especially good results with agglutination and complement fixation. He found, however, that the tests were species specific only

in certain dilutions. When proper precautions were taken as to dilutions, the agglutination test enabled him to differentiate races or strains of algae which had arisen in his pure cultures as a result of varying the nutrient medium and illumination. His final conclusion was that serological methods can be made an exceedingly valuable adjunct in modern algology. Much later GUTTMAN (13), in applying serological technique in phylogenetic studies of the archegoniates, attempted to use *Spirogyra* against the immune sera of several archegoniates. He was unsuccessful because the protein extract (1:200 in 0.85 per cent physiological salt solution) of this alga became turbid after incubation for some time. STEINECKE (35) repeated part of LIESKE's work, using the agglutination, precipitation, and conglutination tests. In harmony with LIESKE, he concluded that the agglutination test is suitable for studies of unicellular algae, but he carried the work much further. He set himself the task of determining whether serological methods could be used to determine phylogenetic relationships in the algae, and, if this proved feasible, to use the results of the test to construct a phylogenetic tree of these forms. He did not use complement fixation because it is especially suitable for diagnosis of species, and he was interested primarily in determining large relationships. Consequently he used the precipitin and conglutination tests. After working out details as to methods of preparing protein extracts, dilutions, etc., he obtained very striking results.

In the meantime serological methods were finding their way to pathological and phytophysiological investigations. The first as well as the most extensive work, although dealing with a phytopathological problem, was not done by phytopathologists, but by soil bacteriologists interested in the bacteria of the nodules of legumes. ZIPFEL (44) seems to be the first one to have made such studies. He showed a serological relation between the strains of the organisms found in the nodules of *Pisum sativum* and *Phaseolus vulgaris* on the one hand, and between *Trifolium pratense* and *Vicia faba* on the other hand, but he found a distinction between these two strains. KLIMMER and KRÜGER (16) employed the agglutinin, complement fixation, and precipitin tests to eighteen distinct strains of legume nodule bacteria. They found the serological characteristics of the

strains to be definite, and were able to correlate them with cross-inoculation of host plants. VOGEL and ZIPFEL (38) showed that legume nodule organisms probably are not one species, that different types can be differentiated by both agglutination and precipitation, and that these methods can be used for differentiation of legume nodule bacteria from other soil bacteria.

STEVENS (36) made a careful study of fifty-five strains of nodule bacteria representing the seven groups of cultivated legumes. His results confirm those of KLIMMER and KRÜGER and others as to host plant groups, and serological properties of the bacteria. In addition he showed that the strains of the same host could be separated into distinct serological groups. SIMON (33), in studying the nodule bacteria of seven genera of Leguminosae, found five cross-inoculating and cross-agglutinating groups. BIALOSUKNIS and KLOTT (4) found that the serological properties of strains remained constant, that more than one serological strain may form nodules on one plant, but that only one serological type is found in each nodule. WRIGHT (42), in studying the *A* and *B* types of *Pseudomonas radicicola* of the soy bean (*Soja max*), found that the strains of the organism are distinct serologically, that the serological characters are constant, and that they are not changed by continued artificial culture or by plant passage. He found, however, that the serological properties of these organisms cannot be used to identify any strain of the organism or the types. FRED, WHITING, and HASTINGS (8), using the agglutination test, found that serologically they could subdivide cultures of legume nodule bacteria belonging to one cross-inoculation group. Upon these differences in agglutination they based their selection of strains used in a study of nitrogen assimilation.

JENSEN (14), an animal pathologist, applied the agglutination test to a study of strains of *Bacterium tumefaciens* isolated from the Paris daisy. He was able to differentiate between the strain he had isolated from the daisy in Denmark and the strain SMITH had isolated from the daisy in America. An excellent piece of work in the application of serological technique to phytopathological problems has been done by NELSON (25), a bacteriologist. He found that he was able, by serological methods, to distinguish between the proteins of a variety of flax resistant to wilt (*Fusarium lini*) and a variety

not resistant to the wilt. Paine and Lacey (28) applied the agglutination test to *Bacillus lathyri*, *Bacterium phaseoli*, and *Aplanobacter michiganense*. Although the first two gave group agglutination, they concluded that these organisms could be differentiated by this method. However, strains were found intermediate between *B. lathyri* and *Bact. phascoli* that agglutinated equally well with the two sera. They suggest that possibly one species had arisen from the other in the tissue of the plant. *Aplanobacter michiganense* failed to agglutinate by either serum or by its own homologous serum. Takimoto (37) reported that serological tests indicate that the bacteria isolated from soft rot of celery, lettuce, and radish belong to the same strain, although the radish organism differs from the others when grown in culture media.

GOLDSWORTHY (10) reports the formation of specific agglutinins, with a titre of 1/10,000, in rabbits following intravenous injection of *Bact. maculicolum* (cauliflower pathogene). He used these agglutinins to determine the presence of *Bact. maculicolum* in the soil.

MEZ (24), in presenting the results of fifteen years' work in the use of sero-diagnosis for determination of phylogenetic relationships of plants, expresses doubt whether serological methods can meet the extreme demands made upon them, by bacteriologists in particular, for the differentiation of strains of microorganisms or of closely related species. He states:

Hier sei nur betont, das eine gewisse Ernüchterung der Bakteriologen bezüglich der Serodiagnostik, die sich mehrfach bis zum Zweifel an der Spezifität der Methode gesteigert hat, darauf zurückzuführen ist, dass zuviel von ihr verlangt werde. Die Unterscheidung z.B. von, "Paratyphus 1,2 und 3" oder ähnlich nächstverwandter Formen geht mit Hilfe der Eiweissdifferenzierung nicht, jedenfalls nicht mit Sicherheit. Ebenso bin ich bezüglich der Ergebnisse von Arzt (Mez, Archiv XIII [1926], S. 117ff.), welcher mit Hilfe der genauesten Dosierung der reagierenden Eiweissmengen bei der Untersuchung der Phylogenie der Gerstenvarietäten Erfolg haben will, noch etwas skeptisch.

Experimentation

The work reported in this paper is restricted to a study of *Bact. campestre*, the crucifer pathogen, and to the bean pathogens *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* which Sharp used in his studies. The value of our results would have been en-

hanced had we been able to include other yellow schizomycetes which are similar to *Bact. campestre* and *Bact. phaseoli*.²

We decided to use first the simplest serological methods (agglutination and precipitation tests), and, if these failed, to resort to the much more complicated complement fixation test. Since we obtained striking results with the agglutination test, we merely applied the precipitation test to determine whether broth cultures of these organisms could be used in making a precipitin test (see accompanying paper by SHARP). The complement fixation test was not used at all.

The cultures of the organisms used were tested for their pathogenicity both before and at the close of the experiment recorded here. Each organism was grown on standard potato dextrose agar slants ($P_{\#} 7.2$) for four days. Even suspensions were then made of each organism in 50 cc. of 0.85 per cent NaCl solution. These suspensions were used as stock antigens for injection of rabbits and production of antisera. Rabbits were used for production of antisera, two animals being used for each organism and as sources of normal serum. Four intravenous injections of the organisms were made into the ear vein of each of the rabbits at intervals of four days, using 0.5 cc. for the first, and 1 cc. for each of the remaining three injections. Ten days after the last injection the rabbits were bled from the heart, the sera prepared, and kept under aseptic conditions until they were used in the agglutination tests.

The cultures used for preparation of the suspensions in the agglutination tests were grown on standard beef extract-peptone-dextrose agar ($P_{\#} 7.2$). The organisms were washed off the agar with a 0.85 per cent salt solution, and then shaken thoroughly in the saline solution and centrifuged. The supernatant liquid was poured off and the organisms were washed and centrifuged a second time, the last suspension in saline solution being used for the agglutination tests. Suspensions of *Bact. campestre*, *Bact. phaseoli*, *Bact. phaseoli sojense* (rough), and *Bact. flaccumfaciens* are centrifuged out of suspension much more readily than *Bact. phaseoli sojense* (smooth). The organisms were grown on different media and washed in saline to take pre-

² This has now been done by LINK and LINK. Paper in manuscript and will appear in BOT. GAZ. 1927.

caution against a positive precipitin reaction with the protein of the potato agar used in preparing the antigen.

The sample protocol of table I shows the set-up used in each experiment: 0.4 cc. of antiserum was added to 0.6 cc. of saline solution (0.85 per cent NaCl), giving a dilution of 1-2.5. From this original dilution the other dilutions were obtained by mixing and then transferring 0.5 cc. of the diluted antiserum from tube 1 to tube 2 which contained 0.5 cc. of saline, from tube 2 to tube 3, and so on, up through tube 10 or 11. After thoroughly mixing the contents of

TABLE I
TYPICAL PROTOCOL OF AGGLUTINATION TESTS

	TUBES											CON- TROL
	1	2	3	4	5	6	7	8	9	10	11	
(Dilution... Antiserum Amount in cc.....	1-2.5	1-5	1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560
Bacterial suspension (antigen) in cc.....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
Saline solution (0.85 per cent NaCl).....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Final dilution of anti- serum.....	0	0	0	0	0	0	0	0	0	0	0	0.5
	1-5	1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120

each tube, 0.5 cc. of bacterial suspension in saline solution (antigen) was added to each tube, thus giving a dilution of 1-5 in tube 1, up through dilution 1-5120 in tube 11. The control in each set-up consisted of a tube containing 0.5 cc. saline and 0.5 cc. of bacterial suspension in saline. After thorough mixing by shaking, the series of tubes was incubated for 1 hour at 37° C. in a Wasserman bath and then placed in a refrigerator for 12 hours. Complete clearing of the suspension by agglutination is indicated by +++, partial agglutination by ++ and +, and the last definitely detectable agglutination by +; ± indicates that there was suggestion of a clumping.

As a control for all series, the experiment was also done using the serum of a normal animal against the suspensions of the organisms tested. Unfortunately one animal was lost, so that there was the normal serum of only one rabbit for a control. The results of the experiments are given in tables II, III, and IV.

Slight agglutination occurred in very low dilutions when normal serum of one rabbit was used against suspensions of the organisms

TABLE II

AGGLUTINATION TESTS USING NORMAL SERUM AGAINST SUSPENSIONS OF BACT. CAMPESTRE, BACT. PHASEOLI, BACT. PHASEOLI SOJENSE, AND BACT. FLACCUMFACIENS

DILUTION OF SERUM	BACT. CAMPESTRE	BACT. PHASEOLI	BACT. PHASEOLI SOJENSE	BACT. FLACCUMFACIENS
I-5.....	++	+	+	+
I-10.....	+	+	+	+
I-20.....	+	±	±	±
I-40.....	-	-	-	-
I-80.....	-	-	-	-
I-160.....	-	-	-	-
I-320.....	-	-	-	-
I-640.....	-	-	-	-
I-1280.....	-	-	-	-
I-2560.....	-	-	-	-
I-5120.....	-	-	-	-
I-10240.....	-	-	-	-
Saline.....	-	-	-	-

TABLE III

AGGLUTINATION TESTS USING ANTISERUM OF BACT. CAMPESTRE AGAINST BACT. CAMPESTRE, BACT. PHASEOLI, BACT. PHASEOLI SOJENSE, AND BACT. FLACCUMFACIENS

DILUTION OF SERUM	BACT. CAMPESTRE	BACT. PHASEOLI	BACT. PHASEOLI SOJENSE	BACT. FLACCUMFACIENS
I-5.....	++++	+	+	+
I-10.....	++++	+	+	+
I-20.....	++++	+	+	+
I-40.....	++++	-	-	±
I-80.....	++++	-	-	-
I-160.....	++++	-	-	-
I-320.....	++++	-	-	-
I-640.....	++++	-	-	-
I-1280.....	++++	-	-	-
I-2560.....	++++	-	-	-
I-5120.....	+++	-	-	-
I-7680.....	+	-	-	-
I-10240.....	-	-	-	-
Saline.....	-	-	-	-

(table II). This probably was due to an idiosyncrasy of the normal serum of this animal, because a test made against *Bact. phaseoli*

with the remainder of the normal serum used by SHARP gave no agglutination at all. Consequently it probably is permissible to ignore the slight agglutination noted in dilutions 1-5 to 1-20, especially since doing so does not essentially alter the data or the conclusions. It would have been better and the results absolutely conclusive if we had, as we are now doing, bled each rabbit before beginning the injections to determine the reaction of the normal serum with the antigen used.

TABLE IV

AGGLUTINATION TESTS USING ANTISERA OF BACT. PHASEOLI, BACT. PHASEOLI SOJENSE, AND BACT. FLACCUMFACIENS AGAINST HOMOLOGOUS ORGANISMS AND AGAINST BACT. CAMPESTRE

DILUTION OF SERUM	ANTISERUM OF BACT. PHASEOLI VS:		ANTISERUM OF BACT. PHASEOLI SOJENSE VS:		ANTISERUM OF BACT. FLACCUMFACIENS VS:		NORMAL SERUM VS: BACT. CAMPESTRE
	BACT. CAMPESTRE	BACT. PHASEOLI	BACT. CAMPESTRE	BACT. PHASEOLI SOJENSE	BACT. CAMPESTRE	BACT. FLACCUM- FACIENS	
I-5.....	++++	++++	+++	++++	+++	++	++
I-10.....	++++	++++	++	++++	++	+++	+
I-20.....	+++	++++	++	++++	+	++	+
I-40.....	++	++++	++	++++	-	++	-
I-80.....	+	+++	+	++	-	++	-
I-160.....	±	++	±	+	-	+	-
I-320.....	-	+	±	+	-	-	-
I-640.....	-	+	-	±	-	-	-
I-1280.....	-	±	-	-	-	-	-
I-2560.....	-	-	-	-	-	-	-
Saline.....	-	-	-	-	-	-	-

The antiserum of *Bact. campestre* (table III) was highly specific for its homologous organism, giving complete agglutination through the dilution range of 1-5 to 1-2560, but gave only a slight (++) or (+) agglutination in low dilutions with suspensions of the heterologous organisms against which it was tested. The titre obtained against the homologous organism was 1-7680.

Table IV shows that when the antisera of *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* were used against suspensions of their homologous organisms, specific agglutination occurred. *Bact. phaseoli* gave a titre of 1-1280, with complete agglutination in dilution 1-5 to 1-40. *Bact. phaseoli sojense* gave a titre of 1-640, with complete agglutination in dilution 1-5 to 1-40. The antiserum of

Bact. flaccumfaciens gave a titre of 1-160 but no complete agglutination at any dilution. *Bact. phaseoli* and *Bact. phaseoli sojense* gave agglutination with *Bact. campestre* in dilutions 1-160 and 1-320 respectively, while antiserum of *Bact. flaccumfaciens* gave agglutination with *Bact. campestre* in dilution 1-20. Consequently in low dilutions, group agglutination was obtained when antisera of *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* were used against *Bact. campestre*.

On the basis of current interpretations of many immunologists, these results indicate that although *Bact. campestre* contains some antigens (proteins) which stimulate the production of the same antibodies as the bean pathogens studied, that is, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*, in addition it contains others which stimulate the production of different antibodies from the bean pathogens. Clearly, therefore, *Bact. campestre* can be differentiated specifically from the bean pathogens on this basis. The group agglutination, shown when antisera of the bean pathogens are used against suspensions of *Bact. campestre* in low dilutions (table III), indicate that *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* contain antigens (proteins) which cause the production of antibodies that react with *Bact. campestre*. The slighter agglutination obtained when antiserum of *Bact. flaccumfaciens* was used against *Bact. campestre* indicates that the former organism does not contain as many antigens in common with *Bact. campestre* as *Bact. phaseoli* and *Bact. phaseoli sojense*. On the basis of these interpretations, *Bact. campestre* is closely related to *Bact. phaseoli* and *Bact. phaseoli sojense*, although it is distinct from them, while it is less related to *Bact. flaccumfaciens*.

Conclusions

1. The agglutination test can be used to differentiate *Bact. campestre* from *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*.
2. Serologically *Bact. campestre*, although distinct, is closely related to *Bact. phaseoli* and *Bact. phaseoli sojense*. It is less related to *Bact. flaccumfaciens*.

3. The biological specificity shown by *Bact. campestris*, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* in their host relations is correlated with immunological or serological specificity.

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RELATION OF DESICCATING WINDS TO FLUCTUATIONS IN ASH CONTENT OF CITRUS LEAVES AND PHENOMENON OF MOTTLE-LEAF¹

A. R. C. HAAS AND H. S. REED

(WITH THREE FIGURES)

Introduction

The vegetation of southern California is exposed to severe desiccating winds during the months of October, November, and December. Their meteorology has been discussed by BLAIR (1), McADIE (4), and CARPENTER (3). Often the winds blow continuously for several days, but at times intermittently, thus giving the trees an opportunity to recover between the windstorms. The hot winds may strip leaves from the trees, wilt, or scorch them. Leaves will recover from the wilted condition unless the wind persists for some time, but scorched leaves are killed without much preliminary wilting (fig. 1).

It has been found that the mature orange leaf has less resistance to excessive water loss than the leaves of many other trees (2). Apparently the stomata of the older leaves have little power to protect against such severe losses. Whether the scorching of leaves is due to an insufficient supply of water in the soil, or to inability of the conducting systems to carry sufficient water to the leaves has not been determined; probably both factors play a part.

Scorched orange leaves may remain attached to the tree for some time and continue to lose water (fig. 1). Leaves which have been severely wilted often have areas killed although most of the leaf recovers (fig. 2). If the dry hot winds are sudden and severe, leaves that are struck directly, in an unprotected grove or in the tops of tall trees, may be killed almost immediately without the loss of very much water. So long as the drying leaf remains attached there is likelihood of a slight loss of moisture through the old leaf. Frequently the wind blows severely for a few hours, causing leaves to become badly wilted, but the subsequent calm permits partial recovery. The

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winds may again resume their activity, and during several days there may be successions of wind and calm. Ordinarily such brief winds do not cause severe injury except to strip the leaves from the tree mechanically.

If the leaves have been killed, new ones arise in the leaf axils. Some important internal action is going on under the influence of the

wind to annul the external action, according to the theory of LE CHATELIER. As the wind blows and evaporates moisture from the leaves, the concentration of salts increases, which serves to raise the osmotic pressure of the cell sap and presumably to reduce the rate of transpiration. It is conceivable that frequent but brief winds of moderate intensity may raise the salt content of the leaf cells to a point where they are better able to withstand prolonged desiccation by subsequent winds. It has been noted that the worst effects are usually produced by severe winds in early autumn.

When the wind ceases and the transpiration rate falls, the wilted leaves regain their turgor and normal salt content. The excessive salts are for the most part redistributed in other portions of the tree or returned to the soil solution.

If leaves are killed soon after the wind strikes them, they lose but little water prior to this and but little salt accumulates within them. If the leaves are killed after the salt accumulation has reached a high level, however, their loss is probably more injurious to the shoots and other portions of the tree, because they are thus deprived of some of their salts.

The loss of calcium through wind defoliation may be inferred from the mottled condition of the next set of leaves (fig. 3); and the



FIG. 1.—Orange twig bearing scorched leaves; photograph shows adherence of leaves a month after they were killed.

loss of organic nutrients such as carbohydrates and proteins may be inferred from the small size of the subsequently formed leaves.

Experimentation

The effect of desiccating winds upon the composition of mature orange leaves has been studied, using material from trees growing in soil in lysimeters 4 feet deep and 8 feet in diameter, with a drainage

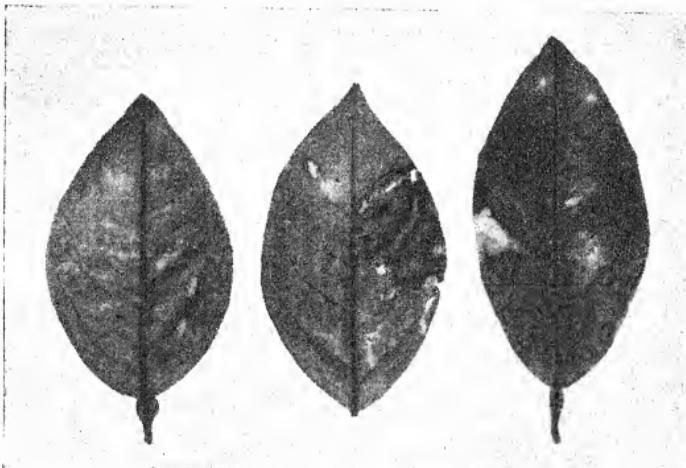


FIG. 2.—Dead areas on orange leaves due to temporary drought conditions; specimens came from trees in pots of soil, but similar injury may be seen in groves not sufficiently irrigated.

pipe from the lowest point of the tank. The soil was carefully irrigated to maintain a desirable moisture content without the use of an excess of water.

The weather records were kindly furnished by the Department of Orchard Management of this Station. Following a period of cool humid weather, a dry wind began to blow on December 9, 1924. The temperature began to rise and the humidity to fall as the wind velocity increased. Some of the data on atmospheric conditions are given in table I. The wind velocity on December 11 at times was more than 25 miles per hour, but the temperature was higher on the

next day and the cumulative effect of the desiccating factors was then reached.

Within an hour certain leaves, struck directly by the wind, were dry as powder, although still attached to the tree. Such leaves were killed so quickly that no appreciable amount of salt accumulated in them. Others were badly wilted even though the soil was sufficiently moist. Mature leaves that were killed and also those that were wilted were collected on December 12, 1924, within a few hours after the wind began. When the wind ceased the wilted leaves regained

TABLE I
CLIMATOLOGICAL DATA FOR DAYS ON WHICH LEAF
SAMPLES WERE COLLECTED

ATMOSPHERIC CONDITION	DECEMBER 1924				
	10	11	12	21	22
Temperature maximum ($^{\circ}$ F.).....	69	73	80	60	64
Temperature minimum ($^{\circ}$ F.).....	41	41	46	32	35
Relative humidity (per cent) at 8:00 A.M.	22	20	19	79
Lowest humidity for 24 hours.....	15	12	16	27	46
Average wind velocity (miles per hour)....	10.1	17.8	11.5
Maximum wind velocity.....	22	26	20	2	4
Hours of sunshine.....	7.17	7.34	8.83	5.92

their turgidity, and on December 22 another sample of mature leaves was taken. Table II compares the wilted leaves of December 12 and the turgid ones of December 22 (uninjured), with the quickly desiccated leaves of December 12 (injured). Analysis was made of the water solubility of the ash constituents of the dry matter, and in some cases total nitrogen determinations were made. The injured leaves, when pulverized and floated on distilled water, appeared to be buoyant for a much longer time than uninjured leaf material. The dry wind probably coagulated the protoplasm of the cells and thus made possible an increased content of air in them, which lowered the specific gravity of the material and therefore caused it to remain at the surface.

The leaf samples were dried at 60°–70° C. Table II gives the results of analyses of the samples obtained from trees grown in tanks of soil that were fertilized with calcium nitrate. On December 12 the

uninjured leaves had accumulated considerable salts and the injured ones very little. The point should be emphasized that this accumulation was not permanent but was dependent upon the occurrence of the wind. The winds did not blow to any extent from December 12 to 22, and the figures for December 22 show that the ash had returned to approximately what it was before the wind began. The point of interest is that there may be a temporary accumulation of ash constituents in the leaves instead of a permanent one, and that

TABLE II

EFFECT OF DESICCATING WINDS UPON COMPOSITION OF VALENCIA ORANGE
LEAVES FROM TREES WHERE CALCIUM NITRATE HAD BEEN APPLIED TO
SOIL; RESULTS EXPRESSED AS PERCENTAGE OF DRY MATTER

	DECEMBER 12, 1924		DECEMBER 22, 1924 Uninjured	DECEMBER 12, 1924		DECEMBER 12, 1924	
	Injured	Uninjured		Injured	Water insoluble	Water soluble	Water insoluble
							Water soluble
Ash.....	14.10	17.03	14.58	6.10	8.11	6.26	10.31
Ash constituents:							
Na.....	0.14	0.14	0.18	0.16	0.03	0.18
K.....	0.60	0.50	0.60	0.04	0.68	0.02	0.53
Ca.....	4.51	5.76	4.83	2.08	2.39	2.26	3.46
Mg.....	0.40	0.42	0.38	0.08	0.31	0.08	0.40
Cl.....	0.03	0.04	Trace	0.03	Trace	0.04
SO ₄	0.40	0.33	0.30	0.09	0.26	0.05	0.26
PO ₄	0.61	0.56	0.54	0.15	0.32	0.16	0.43

the ash content of citrus leaves in the field may depend not only upon their age, but also upon the severity of external climatic conditions. If the winds blew continuously the ash content of the leaves might remain relatively high. It seems logical to conclude that the salt requirement of the leaves must have been satisfied prior to the increase due to the wind; otherwise there would be no such return to the original concentration as is found after a period of calm weather (table II).

The effect of the wind seems to be especially reflected in the calcium content (table II). In uninjured leaves the calcium is always considerably increased by the wind, and if such leaves are killed before the wind ceases blowing it can readily be understood why the shoots should be depleted of soluble calcium for the subse-

quent new growth. This is probably a potent cause of much of the mottle-leaf which occurs on trees exposed to desiccating winds. In spite of the fact that the soil was rich in soluble calcium, the shoots



FIG. 3.—Leaves on orange twig defoliated by desiccating winds: *M*, small mottled mature leaves on shoot whose first crop of leaves was killed by wind; *N*, normal leaves on succeeding cycle of growth; *Y*, young leaves on last cycle of growth, still immature but healthy.

in many cases were depleted of it through the loss of leaves. Since percolates from these lysimeters contained 1300–2500 p.p.m. Ca on September 24, 1924, it seems safe to assume that there was no calcium deficiency in these soils. Fig. 3 shows strikingly the mottled,

dwarfed leaves on shoots which had suffered this type of defoliation. After the trees were adequately protected by windbreaks, the shoots produced healthy large leaves on the next cycle of growth. It seems, therefore, that the trees were unable to acquire sufficient calcium from the soil to produce healthy leaves, at least during the period immediately following defoliation. At that season the new growth of the orange tree seems largely dependent upon the supplies of soluble materials already in the shoots.

The data in table II also show that the increase in salt content of the leaves was due to an increase in the water soluble portion. The increase of water soluble ash from 8.11 to 10.31 per cent is very significant. The uninjured survivors showed an increase in water soluble calcium from 2.39 to 3.26 per cent of the dry matter, but the increase in insoluble calcium was relatively small. The writers have shown (6) that the amount of calcium expressed as a percentage of the ash for leaves, shoots, and trunks was 28.16, 28.01, and 23.51 respectively. Calculated as a percentage of dry matter, this would be 3.93 for leaves, 1.65 for shoots, and 0.55 for trunks. The fractions which were soluble in water varied greatly, being 45.82 per cent for leaves, 15.03 per cent for shoots, and 8.69 per cent for trunks. These data show that the shoot and trunk contain relatively less calcium than the leaves, and, what is still more important, that it is relatively much less soluble in water. We have also shown (5) that orange trees grown without sufficient calcium seemed to be unable to utilize calcium which was present in the trunk and root.

Another aspect of the data in table II pertains to the dynamic equilibrium within the leaf cells. Obviously the salts accumulated as a result of the wind were not largely used by the leaves in the construction of water insoluble compounds, for practically all the increased salt was water soluble. This is in harmony with the suggestion that the increased salt content was of value in protecting the leaves from excessive evaporation.

The conception commonly held regarding the ash content of leaves is that it increases permanently under certain conditions; but so far as we are aware this alternation of low and high salt content (the dynamic equilibrium concept of their inorganic constituents) has not before been emphasized or used in explaining physiological

troubles of leaves such as mottle-leaf. The ash content of citrus leaves subject to the action of winds may fluctuate considerably. During December, 1925, there were some warm but not excessively hot winds of very short duration. Samples of leaves were collected

TABLE III
RELATION OF ASH CONTENT OF CITRUS LEAVES
TO ATMOSPHERIC CONDITIONS

		DECEMBER 16, 1925	DECEMBER 21, 1925	DECEMBER 31, 1925
VARIETY		ASH (PERCENTAGE DRY MATTER)		
Navel orange	A*	Sweet orange 15.04	14.70	15.24
	A.	Trifoliate orange 14.81	14.99	17.03
	B.	Trifoliate orange 15.71	15.83	15.81
Valencia orange	A.	Sweet orange 18.34	17.79	18.67
	B.	Sweet orange 16.62	15.23	16.25
	A.	Trifoliate orange 17.40	16.74	17.35
	B.	Trifoliate orange 17.19	16.94	16.83
	A.	Pomelo 15.13	15.17	15.15
	B.	Pomelo 17.61	14.26	15.15
	A.	Sour orange 15.47	14.95	15.14
	B.	Sour orange 15.77	15.80	18.36
Average.....		16.28	15.67	16.51
Eureka lemon	A.	Sweet orange 16.61	13.95
	B.	Sweet orange 17.35	16.19
	A.	Pomelo 14.55	13.05
	B.	Pomelo 17.19	15.33
	A.	Sour orange 15.91	16.82
	B.	Sour orange 19.79	17.91
Average.....		16.90	15.54

* In each case tree A stood at the north end of the row where it was fully exposed to the wind; tree B was the fifth tree southward and was therefore somewhat less exposed to the wind.

December 16, when moderate winds were blowing, and again on December 21 after a rather tranquil period. A third sample was collected December 31 when the wind again was blowing. The ash content of orange leaves collected on these three days is given in table III, with data on the ash content of lemon leaves on the first

two dates. The leaves in question were as nearly the same age as could be chosen, and successive samples were taken from the same trees. The Navel orange trees were somewhat protected on the north but the other trees were not. In each case sample *A* was collected from the tree on the north end of a row, and sample *B* from the fifth tree southward. It will be seen, however, that there were no very consistent differences between the two sets of samples. The data support the general conclusion of the former determinations, in showing that the ash content of the leaves increased during the period of desiccating winds and that it subsequently dropped. It will be remembered that the winds in December, 1925, were not severe, and the differences in ash content of the leaves is correspondingly less, in comparison with leaves collected in December, 1924.

The power of leaves to change their salt content bears little relation to the rootstock on which the tree was propagated. The ability of the leaves to increase their salt content may be looked upon as favorable to the tree, for it is only during the most severe ordeals that the leaves are lost with disastrous results. The stomata of mature citrus leaves do not actively function; hence if the salt content did not increase as it does, disastrous results would occur more frequently than they actually do.

The ash of fruits of Valencia oranges on the three dates mentioned showed no significant changes, the values found being 4.3, 4.21, and 4.0 per cent of the dry matter respectively. A further indication of constancy in composition was given by a determination of the ash of small fruits from the same trees on June 22, which showed ash amounting to 4.7 per cent of the dry matter. There would consequently be but little chance for the trees to withdraw inorganic constituents from fruits when a new set of leaves was being produced.

An increase in the ash content of the dry matter of mature citrus leaves may be brought about by a long period of warm weather with an inadequate supply of moisture in the soil. A remnant of a citrus nursery was abandoned in 1917 by the Citrus Experiment Station near the Rubidoux plots. Although the remaining trees were left without irrigation, some of them were still alive after about eight years. Early in the spring each year, the full sized leaves went into

a wilted condition upon the advent of hot weather, and remained in this condition until the beginning of the winter rains.

A sample of leaves was collected from the trees on July 29, after several weeks of hot weather. The surface of the soil around the trees was then converted into basins and filled with water to a depth of 3 inches. On the second, sixth, and twelfth days after irrigation other samples of leaves were collected and dried for analysis. Before irrigation the ash of the leaves was 15.52 per cent of the dry matter; two days after irrigation it was 14.39. The two subsequent samples showed very little further change.

TABLE IV
RELATION OF WIND INJURY TO AMOUNT OF INSOLUBLE NITROGENOUS MATERIAL IN MATURE ORANGE LEAVES

DATE 1924	VARIETY	ROOTSTOCK	TOTAL NITROGEN (PERCENTAGE DRY MATTER)			
			Insoluble		Soluble	
			Injured	Un-injured	Injured	Un-injured
December 3..	Navel orange	Sour orange	2.08	1.83	1.17
3..	Valencia orange	Trifoliate orange	1.64	1.48
12..	Valencia orange	Sour orange	1.89	1.64	1.04
12..	Valencia orange	Sour orange	2.00	1.83	0.79	1.10
12..	Valencia orange	Sour orange	2.00	1.81	0.82	0.87

It is of interest that citrus leaves can live through such periods of prolonged and continuous wilting, and that upon irrigation they become turgid so rapidly and lose their increased salt content. A remark upon the difference between wilting and scorching seems appropriate. A wilted leaf will recover when adequate water is supplied from the shoot, but a scorched leaf never recovers. Wilting is not necessarily an intermediate stage of scorching. The blade of the scorched leaf may turn brown and become brittle in a few hours if exposed to desiccating wind. Its color is similar to that of a leaf which has been killed by immersion for a few minutes in boiling water. The behavior of a scorched leaf suggests that its death may be due to coagulation of the proteins of the protoplasm; in that case we might expect to find more insoluble nitrogen in the injured leaves.

Accordingly some of the material was analyzed for nitrogen. The dried material was extracted with water as stated, and the amounts found are shown in table IV. It is clear from the results that there was more insoluble nitrogen in the injured leaves, and it may therefore be assumed that the proteins were coagulated when the leaves were injured.

Summary

1. The effect of desiccating winds upon citrus leaves was shown by their high transpiration and wilting. Analyses here reported show that there was a temporary accumulation of salts in the leaves accompanying excessive transpiration. The excess salt content of leaves which survived the windstorms disappeared during subsequent calm weather. There appeared to be an intimate relation between climatic conditions and salt content of leaves. The increased concentration of salt in the leaves appeared to be due largely to the increase in calcium.
2. The increase in salt content of the leaves was mainly due to an increase in the water soluble portion, especially that of calcium. It appeared that the increased salt content of the leaves was of value in protecting them from excessive evaporation, and emphasized the dynamic equilibrium between the leaf cell and its environment.
3. The loss of calcium through wind defoliation was inferred from the mottled condition of the next set of leaves, and the loss of organic nutrients such as carbohydrates and proteins was inferred from the small size of the subsequently formed leaves. In later cycles of growth the shoots produced healthy large leaves.
4. The larger amounts of insoluble nitrogen found in injured leaves made it probable that the desiccating wind had produced injury by coagulating the proteins of the leaf cells.

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DIFFERENTIATION OF VASCULAR BUNDLE OF TRICHOSANTHES ANGUINA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 366

GRACE BARKLEY

(WITH PLATE VIII)

Introduction

Notwithstanding the great progress of vascular anatomy during the past three decades, but little attention has been given to the development of the vascular bundle, although the development of the other parts of the plant has been known for some time. We are familiar with each step in spermatogenesis and oogenesis, from the archesporial cell to the reduction of chromosomes and the formation of eggs and sperms; but no such series has been described in the development of the vascular bundle, and still less is known of the origin and development of the various markings on the inside of the cell wall.

Morphologists have studied the embryo up to the differentiation of the dermatogen, periblem, and plerome; and vascular anatomists have studied the bundle after differentiation has taken place, but there is a region between the studies of the morphologists and the anatomists which seems to have been neglected by both. It was the original purpose of this investigation to study this neglected region in *Trichosanthes*, and some observations have been made along this line; but the material proved so favorable for a study of the origin of the markings on the spiral and annular vessels, and also of the origin of the bordered pits, that most of the investigation has been devoted to these features.

Material and methods

Trichosanthes anguina, a tropical member of the Cucurbitaceae, seems favorable for a study of the differentiation of the bundle, since the phloem can be recognized in very early stages, and the long slender internodes make it possible to get very effective longitudinal

views. The material used was brought from near Calcutta, India, where it is native, to a station of the Bureau of Plant Industry at Miami, Florida. Seeds were secured from this station and planted in the University of Chicago greenhouse in September. The plants reached maturity late in the following summer, and began to die in October, a period of about thirteen months. The plant is a vine with a long slender angled stem. Specimens grown in the Chicago greenhouse in 6 inch pots reached a diameter of 1.3 cm. and a length of 8-16 m. At Miami, where it grows out of doors and rooted in the ground, it reaches a diameter of 2.2 cm., and, while the vine was not measured, the length was much greater than those in the greenhouse. In both cases the gourd was over 60 cm. in length and 5-7 cm. in diameter. The gourd is used for food in tropical countries, where the plant is widely cultivated for this purpose.

For topographical study, living and prepared sections were made from various places throughout the stem. The rest of the investigation was confined to the first thirteen nodes, where much of the differentiation takes place. The vine at this point seldom exceeds 2 mm. in diameter. The material was killed in a hot solution of mercuric chloride in 50 per cent alcohol with 5 per cent acetic acid. The stains used were combinations of light green and safranin, crystal violet and safranin, and iron haematoxylin. The safranin and light green stains were best for early vacuolization, crystal violet and safranin for the early stages in lignification, and the iron haematoxylin for some stages of the sieve plate.

Topography

The mature stem has five angles and five deep furrows. A cross-section shows an epidermis with glandular hairs, some of which are capped with a single large cell, while others have a multicellular tip. There are also some with pointed tips. Hairs are not found on the older regions of the stem. The stomata are elevated.

Beneath the epidermis at the angles is a collenchyma tissue seven or eight cells thick. Beneath this and also beneath the epidermis between the angles is a layer of chlorenchyma about three cells thick. Next there is a layer of sclerenchyma about three cells thick in the young stem. As the stem grows older the sclerenchyma is found only

on the peripheral side of the bundles. A lysigenous cavity appears in the central part of the stem about 2 cm. from the tip, but it later becomes closed by the growth of the bundles. It is also found in the stem near the roots and in the petiole of the tendril.

There are ten bundles in the stem, five large ones occupying the angles and five small ones more deeply placed. The inner bundles grow more rapidly at first, and at a certain stage are much larger than the angle bundles. All the bundles are bicollateral, with the exception of two of the inner ones, and even these become bicollateral near the root. These two bundles are also peculiar in that they show no protoxylem except near the root.

There is fascicular cambium in the outer phloem. Cambial activity also produces lateral phloem in the older parts of the stem. This lateral phloem is not continuous along the side of the bundle, but appears here and there at the side of the xylem. One stem observed had a small bicollateral bundle produced by this cambium. Here the cambium was next to what corresponds to the inner phloem, and there was also cambium in the outer phloem of this bundle. Besides these patches of phloem there are independent patches here and there in the periphery.

The petiole usually has nine bundles, all of which are bicollateral except the two upper ones in the angles of the groove. The petiole of the tendril has five angles, in each of which is a bicollateral bundle. In the male peduncle there are seven to nine bicollateral bundles, but in the female peduncle there are as many as fifteen. There is great variation in the number of bundles in the petioles and peduncles, the number in the petiole varying from nine to eleven, in the tendril from five to seven, and the number in the male peduncle from seven to nine.

In tracing the differentiation of the bundle, it is very desirable to know the position of the young bundle in the topography, since the two bundles which are not bicollateral differentiate later than the others.

Differentiation of bundle

In tracing the development of the vascular bundle, it would be logical to begin with the undifferentiated meristem at the stem tip, and follow the gradual divergence of the meristematic cells until the

characteristic features of the mature bundle are attained; but practically, with the problem in its present condition, this method would be difficult and perhaps uncertain. Consequently the writer has adopted a method similar to that chosen by GREGOIRE when he began his series of investigations upon the structure of chromatin. Instead of beginning with the resting nucleus and tracing the chromatin through a continuous cycle, he began with the fully formed, easily recognizable chromosome and traced the changes which occur from that point on to the resting condition.

PROTOXYLEM

The present study of the bundle begins at a stage in which the xylem and phloem portions are recognizable, but still more or less parenchymatous. A cell, which is to form a part of the spiral vessel of the protoxylem, will serve as a starting point from which changes will be noted as one proceeds toward the meristem and also toward the mature, spirally marked vessel.

About 3 mm. from the stem tip a conspicuous feature of the longitudinal section is one or more rows of large cells (fig. 1). These are parenchymatous and are very large compared with the adjacent parenchymatous cells. The lower cell is typical of this type. It has a length of 81.5μ and a width of 9.8μ . The nucleus is also very large. Except in the size of the cell and nucleus, the cell is undifferentiated. Most of the interior of the cell is occupied with a large vacuole with protoplasm lining the cell wall, surrounding the nucleus, and connecting the latter with the peripheral cytoplasm. Toward the tip these cells become shorter and shorter, as is seen in the cells above the one described. They also become narrower, the nucleus smaller, the cytoplasm denser, and the cells similar to the neighboring ones in appearance.

In another cell (fig. 2) farther from the tip than this one, considerable differentiation has taken place. The nucleolus has disappeared and the outline of the nucleus is irregular, showing that it has begun to disintegrate. The central vacuole is very large, but strands of cytoplasm still remain, connecting the nucleus with the periphery. The peripheral cytoplasm has taken the spiral arrangement seen in the thickenings of the spiral vessel, with bands of more

finely vacuolated cytoplasm alternating with bands which are more coarsely vacuolate. A portion of this cytoplasm, more highly magnified, is shown in fig. 4, where the magnification is great enough to show the nature of the bands. They are composed of cytoplasm with vacuoles so small and numerous that the band looks homogeneous under a high power dry lens. Often there is only a single row of larger vacuoles between two bands, but sometimes there are two rows of larger vacuoles, as shown between the upper two bands of fig. 4. After the finely vacuolated portion of the spiral has become the thickened lignified band of the spiral, the protoplasm between the two rows of larger vacuoles appears as a line intermediate between the bands of the spiral (fig. 5*l*). In an earlier stage than that of fig. 4 the cytoplasm between the rows of vacuoles appears as faint granular striations which are seen only with careful focusing (fig. 3). At first sight these striations look like rows of granules, but they are the result of regularly placed vacuoles, and the apparent granules are the denser portions of cytoplasm between them. Unless well fixed and stained, the cytoplasm at this stage looks like that shown in fig. 1, where the arrangement into bands has not yet begun. The faint striations (fig. 3) become the finely vacuolated bands (fig. 4), which later become the lignified bands of the spiral thickening (fig. 5).

In fig. 6 the length of the individual cells that contributed to the formation of the spiral vessel can be seen because the end walls are not yet completely absorbed. The length of the middle cell in this figure is 97.8μ . While the spiral band in this cell is less reddened with safranin than in the vessel below, showing less lignification, the band is almost as large in cross-section, and the cell itself has the same diameter as the cell below. It seems safe to conclude that the differentiation is only in the degree of lignification.

While the cell just above this one is the next adjacent cell, it is considerably less differentiated. The spiral band is definitely outlined but is not at all lignified. Vacuoles are still present, the spiral band is quite granular in appearance, and, although much thicker than the cytoplasmic band in fig. 2, it is not so much thickened as in the cell below.

The three cells in this row, in which the end walls have not broken down, differed widely in the differentiation of the spiral. A strik-

ing feature of the development of the spiral at this stage is that there is no gradual advance in passing from one cell to the next, but a sharp difference in the degree of differentiation between adjacent cells. Later, when the transverse walls have broken down, the development of the spiral becomes uniform throughout the vessel. That these stages are passed through very quickly is shown by the fact that they are seldom found, but when they are seen they always present this sequence of development.

There are three cells between *a* of fig. 6 and the parenchymatous cells of the type of fig. 1, near the meristem end of the row. The third cell above *a*, adjacent to the parenchymatous cells, while much less differentiated (having flatter bands with more vacuoles than the two cells between it and cell *a*) is more differentiated than the cell in fig. 2, for its nucleus has broken down and disappeared.

The annular vessels are formed from cells much longer and narrower than those which contribute to the formation of the spiral vessel. The cell in fig. 7 has a length of 166.6μ and a width of 7.8μ . This cell also has a very long nucleus with a length of 26.9μ and a width of only 4.3μ . The nucleus has several nucleoli and the outline is even, showing that the nucleus has not yet begun to disintegrate. There is here a large central vacuole occupying most of the cell, but cytoplasmic strands still connect the nucleus with the peripheral cytoplasm.

The peripheral cytoplasm, instead of having rows of small vacuoles, as seen in the young spiral vessel, has larger vacuoles extending entirely across the cell. In the upper part of the cell there is no definite arrangement of cytoplasm showing any differentiation, but in the lower part there are large vacuoles with bands of cytoplasm between them. The cytoplasm is denser where it borders upon the larger vacuoles, which, being close together, crowd the denser layers of cytoplasm together, thus forming bands. Above the nucleus is a single band of cytoplasm. Just below this cell is one which is well differentiated, with a series of short loose spirals and rings. Although not yet completely lignified, the cross area of the thickenings is about as great as in the mature vessel.

The protoxylem has several kinds of vessels, the annular, loose spiral, and close spiral. While the annular vessel, just described, was

first differentiated with the formation of rings, after the close spiral near it was quite well differentiated and partially lignified, it reaches complete differentiation sooner than the spiral vessel. At that level of the stem where the annular vessel first seems completely lignified, the spiral thickenings are still stained purple with crystal violet and not nearly so red with safranin, showing that they do not lignify as rapidly as the thickenings of the annular vessel. The thickenings of the latter also have a greater cross area at this time.

Some of the thickenings of the young close spiral have a faint line between the spirals, as if a deposit of the last remaining vacuoles had been left between the main coils. These faint lines doubtless are due to the weak band of cytoplasm between two rows of vacuoles, as already described and illustrated in figs. 4 and 5. Many of the thickenings have a clear streak through the center before complete differentiation, giving the thickening a double appearance. After differentiation these coils are heart-shaped in cross-section, and in some there is even a complete separation of the two parts for short distances, making a slit in the spiral. Some of the loose spiral vessels have two coils in parts of the vessel, each coil being about as far distant as the coils of a single loose spiral vessel. Perhaps this was caused by the same process, only proceeding further so as to make two separate coils. The thickenings of the mature vessel have two regions in cross area, a central area surrounded by an outer border. The outer area seems to have the greater lignin content.

When a spiral protoxylem vessel is adjacent to a pitted metaxylem one, about the time the metaxylem begins to pit, several small vacuoles become visible between the coils of the spiral (fig. 5). These enlarge and lengthen and take a definite arrangement. They become the elongated pits between the coils of the spiral. In its younger stages this pit is occupied by a row of small vacuoles.

METAXYLEM

Although the protoxylem is the first element of the bundle to differentiate, the metaxylem is far more conspicuous. Even after the protoxylem vessels are lignified, the metaxylem cells, although large and still elongating, are thin walled, without any traces of the markings which characterize the mature vessel. They present a greater va-

riety of form than is found in the protoxylem. In the transverse section of the young bundle there are several cells of very large lumen, and these are the large cells of the metaxylem. In longitudinal section the diameter of these cells exceeds the length. Other cells of the metaxylem with smaller diameter are very much longer, but by breaking down of transverse walls both types develop into vessels. Beside these there are smaller parenchyma cells which surround the vessels. Those parenchyma cells surrounding the larger vessels are irregular and sinuous in outline, and those surrounding the smaller vessels have long straight walls.

The first cells of the metaxylem to differentiate are not those of the largest diameter, but the long cells of considerably less diameter. In these the markings are scalariform, with pits so long that in some places they give the impression of a spiral. This cell was formed under conditions more nearly like those which prevailed while the protoxylem was being differentiated, and the markings naturally more nearly resemble those of the protoxylem than do the markings of the other metaxylem vessels which are laid down later.

The next cell to differentiate is intermediate in size between the scalariform cells and the cells of the largest lumen. This intermediate type is comparatively short, the length being only two or three times the diameter in some cells; but there are some cells of this type with a length seven or eight times the diameter. These have long pits, although not so long as those of the scalariform vessel. The last of the vessels to differentiate is the one formed by the large cells mentioned, which are still thin walled when the walls of the other vessels have thickened.

The character of the markings on the metaxylem is different from that on the protoxylem, but the process of formation is very much the same, and the difference is probably due to the different conditions under which the thickenings are laid down.

The scalariform vessel resembles the spiral so closely in its markings that it seems safe to conclude that the thickenings were formed in almost the same way. The thickened band is a little broader and flatter. The pit spaces in early stages are filled with tiny vacuoles, giving the same granular appearance that was found between the coils during the early stages in the formation of the spiral.

The next cell to differentiate bears a similar resemblance to the scalariform type that the scalariform one does to the spiral. Probably the resemblance is due to the fact that two types are formed under somewhat similar conditions. In the same way this last vessel to differentiate would resemble the scalariform, because the conditions which prevail during the formation of the markings are somewhat similar. The thickening is quite evenly distributed over all parts except the pits. These are elongated and in some cells they are so long as to give the cell a scalariform appearance. The pits are formed and the walls are quite thick before the nucleus begins to disintegrate. The lateral walls are slightly lignified when the end walls break down.

The pits of the largest cells vary in shape from elongated to round, and are closely crowded, while the pits of the surrounding parenchyma are small and slightly elongated.

The vacuoles of the young cells of the metaxylem do not have as definite an arrangement as they had in the protoxylem vessels, but they are arranged somewhat in such horizontal rows as are found in the elongated pit markings. These, however, are not as definite in arrangement as in the protoxylem. According to STOVER,¹ the first thickening of the desmogen strand is laid down in pitted form, and these vacuoles cause the cytoplasm to line the wall in the same pattern as the first thickenings. So far as the pitting of the metaxylem is concerned, the writer's observations agree with those of STOVER; but as to the origin of the spiral and annular markings of the protoxylem, the development in *Trichosanthes* differs from his account. When the pits are first formed they are shallow and not at all bordered, but as more and more material is deposited in thickening, it overarches the original pit and forms the border. This is the origin of the bordered pit in *T. anguina*, and may be true for bordered pits in general.

PHLOEM

It has long been known that the sieve tube and companion cell of the Cucurbitaceae are very large. In *Trichosanthes anguina* these elements are not only large but are differentiated very early, so that it is very easy to distinguish the xylem and phloem elements in a

¹ STOVER, E. L., The anatomy of *Calamovilfa*. Ohio Jour. Sci. 24:169-178. 1924.

young bundle. The companion cell is cut off from the sieve cell even before the metaxylem begins to show any markings.

The sieve tube in the mature stem is very long and narrow, with a length varying 150-700 μ and a width of 17-30 μ . The tube continues to lengthen long after it is differentiated. The sieve cell can be recognized with certainty within 1.5 mm. from the stem tip, for at this distance from the tip it has cut off the companion cell, and perforations can be distinguished in the sieve plate (fig. 9). The nuclei persist until the sieve plate reaches its mature condition. In the early stage mentioned, the shortest cell in the figure has a length of 12 μ and the longest a length of 27 μ . The protoxylem at this level is differentiated with markings, but they are not lignified and the metaxylem shows no traces of markings.

The sieve character of the sieve plate can be recognized even before the companion cell is cut off. This is proved by the fact that the perforations can be seen in young stages between adjacent companion cells. On the sieve tube portion the plate thickens unevenly, the thickening being laid down faster on the side toward the root. The thickening process continues for a long time and does not reach its maximum until the nucleus has disappeared.

The early formation of the companion cell makes it easy to recognize the phloem region. The companion cells do not lengthen enough to keep pace with the sieve tube but divide transversely, making a row of companion cells for each sieve tube. They are filled with a very dense cytoplasm containing many large vacuoles.

Since eight of the ten bundles are bicollateral, the stem has a large proportion of phloem. Lateral phloem containing sieve tubes is also found at the sides of the xylem in the older parts of the stem. The phloem is composed of sieve tubes, companion cells, and parenchyma cells, the latter being long and slender.

CAMBIIUM

The cambium is not a persistent part of the desmogen strand, but is a new formation arising between the xylem and phloem, just as phellogen arises in the cortex (fig. 12). The cambium does not appear until the xylem and phloem are easily recognizable, as shown in fig. 12. The cells of the cambium have a denser protoplasm than

that of the surrounding cells, and are considerably elongated. Both the cambium cells and the phloem parenchyma have their end walls very little inclined.

Cambium is found between the xylem and the outer phloem, and in a very small amount between the xylem and the inner phloem. It is also found along the side of the xylem of the bundle in the older parts of the stem. This lateral cambium produces a phloem, and even a small bundle with both xylem and phloem was produced by this cambium in one specimen observed. Traces of interfascicular cambium are found near the nodes in stems about 3 mm. in diameter, but there is no interfascicular cambium in the other parts of the internode.

There are patches of tissue in the peripheral parts of the older stem where meristematic activity sets up. This may be because the stresses upon *Trichosanthes*, as a climbing vine, cause traumatic activity to set up as a traumatic response.

Summary

1. *Trichosanthes anguina* is favorable for a study of the differentiation of the vascular bundle, because the phloem can be recognized very early and the long internodes make it easy to get straight views of the differentiating structures.

2. The spiral vessel of the protoxylem in its early stages has bands of peripheral cytoplasm which precede the spiral markings and have the same arrangement, and become the basis of the lignified spiral. The position of the cytoplasmic bands is determined by rows of vacuoles in the cytoplasm immediately preceding and during the formation of the cytoplasmic bands. The process of the early differentiation of the marking is a very rapid one. The position of the bands of the annular vessel is also determined by vacuoles, but the determining vacuoles are much larger than those of the spiral vessel.

3. The young cell of the metaxylem also has vacuolated peripheral cytoplasm which precedes the formation of its markings. The scalariform vessel, composed of long cells, is the first to differentiate; the vessel composed of shorter cells with elongated pits is the next to differentiate; and the vessel composed of short cells with large lumen marked by round or oval bordered pits is the last to differentiate.

4. The sieve tube differentiates very early. It cuts off the companion cell after the perforations have been formed in the sieve plate. The companion cell may divide transversely several times, so that there may be as many as six or seven along a single sieve cell. The callus of the sieve plate is much thicker on the side next to the root.

5. The cambium does not appear until the xylem and phloem are easily recognizable, and there is scarcely any interfascicular cambium, except near the nodes. Lateral phloem is derived from a cambium which appears still later.

The writer wishes to express appreciation to Professor C. J. CHAMBERLAIN, under whose direction this investigation was carried on.

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EXPLANATION OF PLATE VIII

FIG. 1.—Cell row which will contribute to formation of spiral vessel; $\times 640$.

FIG. 2.—Arrangement of vacuoles in cytoplasm in early formation of spirals, s, coils of young spiral; $\times 1330$.

FIG. 3.—Earlier stage than that shown in fig. 2; $\times 1330$.

FIG. 4.—Portion of cytoplasm in fig. 2, enlarged from camera drawing, showing nature of spiral bands; $\times 5320$.

FIG. 5.—Vacuoles between coils of spiral vessel in early stages of pit formation; $\times 1330$.

FIG. 6.—Cells of spiral vessel before end walls are absorbed, showing difference in differentiation; $\times 580$.

FIG. 7.—Cell contributing to formation of annular vessel, showing early stage in formation of ring; $\times 1330$.

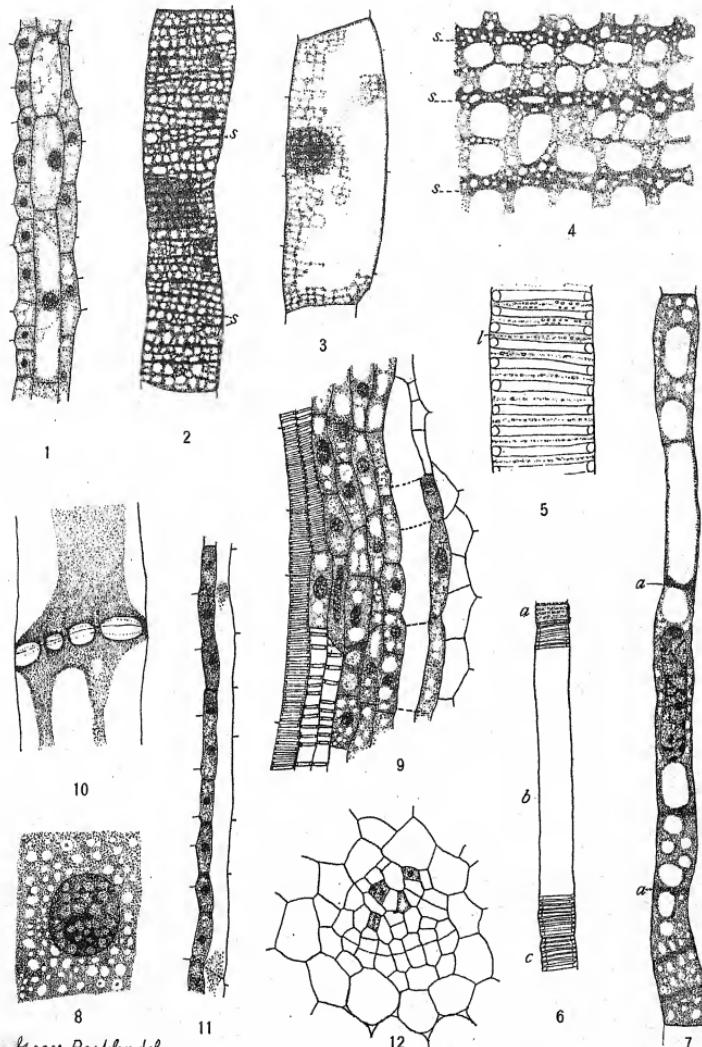
FIG. 8.—Vacuoles in peripheral cytoplasm of young metaxylem cell; $\times 1330$.

FIG. 9.—Young sieve cell and companion cells, showing sieve plate extending between adjacent companion cells; $\times 640$.

FIG. 10.—Sieve plate showing greater thickening on side toward root; $\times 1330$.

FIG. 11.—Sieve tube with row of companion cells; $\times 580$.

FIG. 12.—Origin of cambium in young bundle after other elements are recognizable; $\times 340$.



Grace Barkley del.

BARKLEY on TRICHOSANTHES



DISTRIBUTION AND ABUNDANCE OF SPARTINA
MICHAUXIANA AT DOUGLAS LAKE, CHE-
BOGAN COUNTY, MICHIGAN¹

F. C. GATES, EDITH C. WOOLLETT, AND E. P. BREAKY

(WITH TWO FIGURES)

Introduction

From the standpoint of rapidity of spread in a new habitat in the Douglas Lake region, one of the most interesting plants has been *Spartina Michauxiana* Hitchcock. The activity of this plant, which is nominally a prairie species, had been observed for several years when the appearance of OLIVER's² paper on the colonization of mud flats in southern England and northern France by *S. Townsendii* made it seem rather worth while to record the facts in regard to *S. Michauxiana*.

Just exactly when *Spartina* first made its appearance in the Douglas Lake region is not known, but old lumbermen say that it was not present anywhere at the time the region was lumbered in the 1880's. Presumably it made its appearance in the early years of the present century, possibly at the time when a grade was established for a lumber railway. At any rate, when first observed it was present only in a very few places along this grade and the southwest corner of Douglas Lake, where the grade runs but a short distance from the lake. It has now spread to nearly all parts of the shore of the lake in varying abundance. Since being under observation, for a time *Spartina* extended its patches along the grade, but when the grade was abandoned as a road, the encroachment of the forest with its attendant shade eliminated the plant there. Along the lake, however, it has been spreading vegetatively from year to year in a rather remarkable way. In different years the colonization of new places has

¹ Contribution no. 261 from the botanical laboratory of Kansas State Agricultural College, and a contribution from the Biological Station of University of Michigan.

² OLIVER, F. W., *Spartina Townsendii*; its mode of establishment, economic uses and taxonomic status. Jour. Ecol. 13:74-91. 1925.

been studied, particularly in the Sedge Point region. The plant has made headway against all strand types of native vegetation, but is limited in advancing far from the lake by any vegetation that is capable of shading it. The present paper is the result of the detailed study

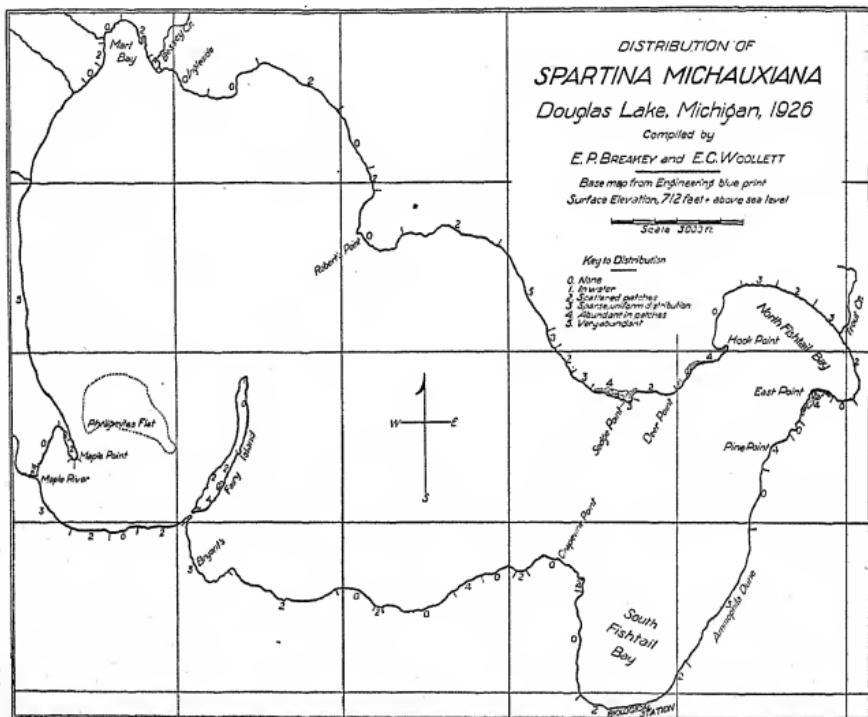


FIG. 1.—Map showing distribution of *Spartina Michauxiana* at Douglas Lake, Michigan, 1926; by E. P. BREAKY and E. C. WOOLLETT.

of the distribution and characteristics of the plant made especially by the junior authors during the summer of 1926.

Douglas Lake is one of several larger inland lakes lying in the Cheboygan River drainage system at the northern end of the lower peninsula of Michigan. Douglas Lake lies entirely in glacial deposits, but it seems certain that the depression existed prior to the last retreat of the glacier, which filled it with small lobes of ice that, melting

more slowly than the main ice sheet, prevented heavy deposition.³ The land surrounding the lake is all of glacial origin, and the shore line is composed primarily of sand with brief stony stretches. With a surface area of 14.92 sq. km. the lake has a perimeter of 24.97 km. Prevailing winds for the region are from the northwest, except for the months of September, October, and December, when the direction is from the southwest. The main axis of the lake is approximately northwest-southeast, with a total length of about 7 km. The P_h reading of the surface waters of the lake varies but slightly from 8.6. The annual precipitation is about 73 cm.⁴ The entire region lies in the transition area between the northeastern coniferous forests and the deciduous forests of the south. The shore of the lake is narrow, with small projections and indentations, especially along the south. Small dunal areas, especially the *Ammophila* dune (fig. 1), are present where the prevailing winds have piled the sand, and vegetation has become established. At various points around the lake small bluffs arise. The shore may be divided into shoal, lower, middle, and upper beaches, and upland vegetation areas. In protected coves, where conditions are favorable for suitable development and growth, the associations of the beach inland from the water include the *Scirpus validus*, *S. americanus*, *Eleocharis palustris*, and *Iris* associations, and the invading *Spartina Michauxiana* meadows.

Present distribution

The distribution of *Spartina Michauxiana* on the shores of Douglas Lake ranges from scattered isolated bunches of a few plants to rather large meadow-like zones, mostly on the lake shore at the edge of the upper beach, although in two places on the lower beach it was found growing out actually into the water. At Maple Point plants were growing in the water, but not where water would be expected to cover them all the year. A layer of aerenchymatous tissue was present in the anchorage roots, but not in the rhizomes of those plants growing in the water. The rhizomes adapt themselves to an

³ SCOTT, I. D., Inland lakes of Michigan. Mich. Geol. and Biol. Survey, Publ. 30. Geology Series 25. 1920.

⁴ For the detailed figures of meteorology of the Biological Station, see GATES, F. C., Meteorological data, Douglas Lake, Michigan. Mich. Acad. Sci. Arts, and Letters 4:475-489. 1924; and other papers referred to there.

average depth of about 8 cm. If they are buried they grow up to meet this level; if the sand is removed from around them they grow down to the level. At no point in the survey were seedling plants found, nor have they been noticed in previous years, apparently indicating that reproduction here is entirely vegetative. On the shore west of Bryant's hotel near the end of Fairy Island, five of the plants were found on the lower beach near the water's edge. Everything would indicate that these plants had recently been torn loose from some section of the lake shore to the northwest, and had been carried to this spot by the wind and waves and cast upon the beach. Three of the plants were well established near the water's edge. The roots of a fourth plant had been covered with sand by the waves, but it still remained in a leaning position. The fifth plant lay on the beach at the water's edge, with only a few of its roots covered. This indicates a method by which the plant is distributed around the lake shore. On Deer Point, where *S. Michauxiana* is well established, it was also found growing out into the water, invading the *Scirpus americanus* association. As 1925 was a year of low water and in all probability the *Spartina* was not then covered, it remains for the future to decide whether or not the water will be an eliminating factor. In the vicinity of Maple River in sloughlike places *Spartina* is entirely absent, although *Calamagrostis canadensis* and *C. inexpansa* are well established. In contrast with the nearly pure sand of the lake shore, the soil near Maple River is of a peatlike character.

Upon the lower and upper beaches, especially along the shore between Marl Bay and Maple Point, *Spartina* has invaded the *Eleocharis palustris* and *Iris* associations, and has established itself in abundance. In several places in this area *Myrica Gale* has shaded out the *Spartina* at the foot of the ice ridge, but on the ridge itself beyond the shaded area it still is growing. In other spots *Spartina* meadows of varying width were found competing with *Scirpus americanus* and proceeding toward the water's edge. In clearings near Bryant's hotel isolated stands are invading the *Scirpus americanus* and *Potentilla Anserina*, and the *Spartina* would be well established were it not for human interference. At Sedge Point three beach pools have been formed by sand bars, thrown across the point by the winds which blow along the shore. On the small sand dune skirting the

pools *Spartina* is quite abundant and is advancing toward the pools, competing with the *Iris* association. On the small dune behind the pools *Spartina* is somewhat well established, and has displaced the native *Iris* association. On the broad marly shoals found between Robert's Point and Bessey Creek, that are subjected regularly to very severe ice work and wave action, it is difficult for the *Spartina* to hold its own in competition with the *Scirpus* and *Eleocharis* associations.

The amount of *Spartina* on Maple Point is probably lessened because of severe ice action, as only scattered patches of the plant were found, although vigorous growth for the year was at its optimum. Ice work has cut back both the *Spartina* and *Scirpus* on Deer Point, which is not too well protected. Between Sedge Point and Bessey Creek the distribution of *Spartina* is very sparse and scattering, due to the severe ice and wave action to which this area is regularly subjected. This is shown by the fact that in the vicinity of Robert's Point numerous specimens of *Acer rubrum* and *Populus balsamifera* have been undercut and overturned by ice and wave action. In this same strip of shore, ice has cut away whole shoal associations, leaving only a brown carpet of matted roots and rhizomes. At East Point the *Spartina* is located primarily upon the dune fringing the northern side of three small beach pools, formed by winds blowing along the shore and offshore winds. Very severe ice action is noticeable at this point on the northwestern shore. In the area between Bryant's hotel and the Biological Station there is a long stretch of stony shore, where water-washed rocks and gravel with the help of ice and wave action keep *Spartina* from developing. Five good patches with sand areas between show that, provided eliminating factors became less severe in action, the *Spartina* would probably advance rapidly. The north and east winds are the more important on this shore, because of the protection from the westerlies offered by Fairy Island.

Shade is an eliminating factor that is especially noticeable in the cove to the north from Hook Point, and the eastern side of Grapevine Point. In such places *Spartina* is wholly absent. In protected coves where the water is shallow and wave and severe ice action are minimum factors of elimination, the plant is well established provided shade is not an eliminating factor. A survey of Fairy Island

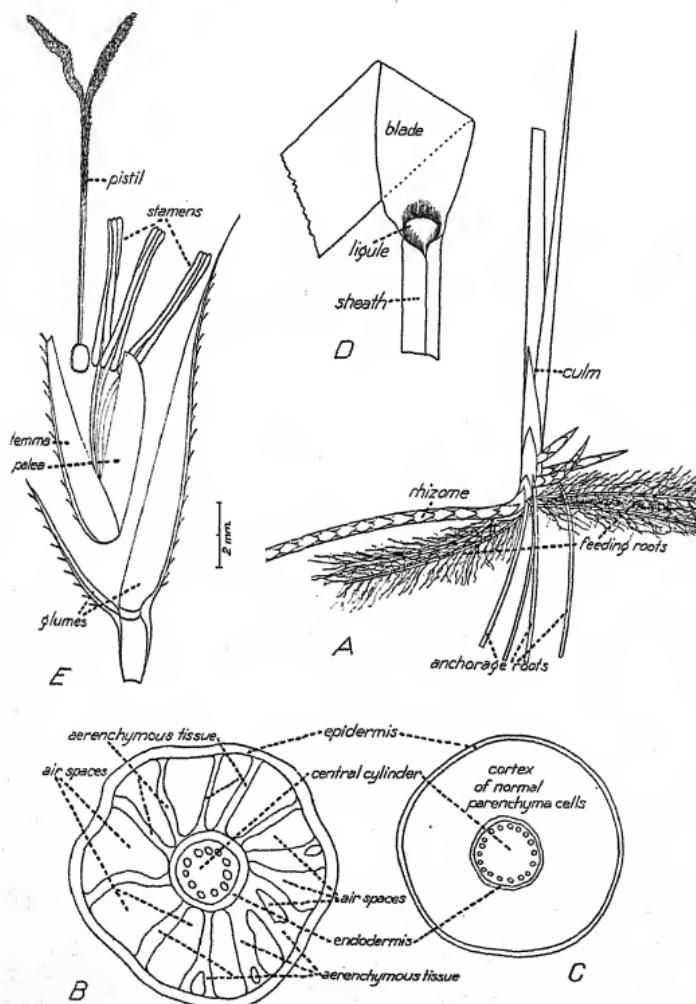


FIG. 2.—Drawings of *Spartina Michauxiana* showing: A, basal part of plant, $\times 0.5$; B, C, diagrammatic cross-sections of anchorage roots (B) and ordinary roots (C); D, detail of part of leaf enlarged; E, details of spikelet (scale shown); by E. P. BREAKEY.

showed that on the eastern shore shade is the principal eliminating factor, with ice action as secondary. Where sufficient sunlight is afforded in this area large patches of *Spartina* are found. In several places near Maple Point where *Myrica Gale* has grown out to the water *Spartina* has been shaded out.

As a dune holder *Spartina* compares well with other plants at Douglas Lake. Its ability to hold sand is best shown at Sedge Point, where a permanent *Spartina* dune is developing. *Ammophila arenaria* is the best dune holder in the region, but is of little importance at Douglas Lake because of the lack of suitable conditions for its dune-developing capacities. Upon the one *Ammophila* dune at Douglas Lake *Spartina* and *Elymus canadensis* are the only grasses competing with *Ammophila*. *Spartina* is becoming very well established on this dune, which has an average height of 2 m. above the level of the lake. At various places, especially along the eastern shore of the lake, *Spartina* is holding small seasonal dunes. The structure of the plant appears to be well adapted to living in shifting sand provided it is not too high. The ability of the rhizomes to adjust themselves to changing levels fits *Spartina* for such a habitat, and in this region its economic importance is primarily that of a dune holder.

Late in June 1926 several sods of *Spartina* were removed from their habitat on the lower beach and transplanted upon Phragmites Flat, in about 12-15 cm. of water. The flat is a broad gravel bar in the lake and shows above water only during low water years. Up to the time of the transplanting of *Spartina* upon the flat, *Phragmites communis* was the only grass established there, where it followed the natural depression around the southern edge. At the end of the summer the flat was again visited to determine how the *Spartina* had fared in its new habitat. The plant had become established during the summer and new shoots were pushing up from rhizomes growing out through the sand and gravel under water.

On the whole, although *Spartina Michauxiana* is well established in certain areas around Douglas Lake, the eliminating factors of shade, ice, and wave action appear to control the distribution at all points.

Systematic study

A systematic study of the plant itself was made: Culms 72-(122)-143 cm. high. Leaves 2.8-(5.5)-9.1 dm. long, tapering to a very slender point, keeled, flat, but quickly involute in drying, smooth except the margins. Spikes 10-(14)-18, scattered, spreading, 4-7 cm. long, rachis rough on margins; glumes serrulate-hispid on keel, the first finely hispid and serrulate on inner margin, acuminate, and shorter than the floret; the second finely hispid and tapering into a serrulate-hispid awn 2-5 mm. long; lemma 7-9 mm. long, finely hispid and with a serrulate scabrous midnerve which abruptly terminates below the emarginate or two-toothed apex.

Comparing this with the description given in GRAY's manual brings out the fact that the *Spartina Michauxiana* in the Douglas Lake region is in all respects a smaller plant, less rank, with the first scale shorter than instead of equaling the floret, the awn decidedly shorter, and with the glumes and lemma, except for the keel, finely hispid instead of glabrous. Studies later at the Herbarium of the Field Museum of Natural History, and field studies in northeastern Illinois and eastern Kansas tend to confirm the impression. HITCHCOCK⁵ states, however, that the description in the manual was too closely drawn, and does not give sufficient leeway to the variation exhibited in specimens, so that it is perhaps best to consider the Douglas Lake plants (and also specimens from lakes in northern Wisconsin and Minnesota) as an ecological variation or ecolog (arenaria) of a prairie plant in a northward or northeastward advance upon sandy shores of northern lakes, with perhaps a further suggestion of the possibility of having a new species in the process of evolution, thus meriting further attention.

Summary

1. The distribution of *Spartina Michauxiana*, nominally a prairie plant, on the shores of Douglas Lake, Cheboygan County, Michigan, ranges from scattered isolated groups of a few plants to rather large meadow-like zones.

2. The habitat of the plant is that afforded by the lake shore, where it grows typically on the upper beach in front of the upland

⁵ In a personal letter to the senior author, after examining a series of specimens from the Douglas Lake region.

vegetation, although in two places it was found on the lower beach growing out actually into the water.

3. The eliminating factors of shade, ice, and wave action seem to control the distribution at all points.

4. *Spartina* is of economic importance in the region primarily as a dune holder in situations not too far above the lake level.

5. The plants of *Spartina Michauxiana* in the Douglas Lake region, although they differ in several particulars from the standard descriptions, at present favor suitable extensions of the description to cover these plants as ecological variations rather than consideration as a new species.

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FURTHER STUDIES ON GROWTH OF CHLORELLA
AS AffECTED BY HYDROGEN-ION
CONCENTRATION
ALKALINE LIMIT FOR GROWTH
F. B. WANN AND E. F. HOPKINS¹
(WITH THREE FIGURES)

In earlier papers (1, 2) it was shown that in a highly buffered culture solution containing calcium, the true effect of the H-ion concentration on the growth of *Chlorella* sp. was masked by the lack of available iron in the more alkaline cultures. This condition was produced not alone by chemical precipitation of the iron, but largely also by its adsorption on the amorphous precipitate of calcium phosphate present in all the solutions more alkaline than P_H 5.7. It was further shown that, while the presence of sodium citrate would prevent the chemical precipitation of iron at all H-ion concentrations where this otherwise occurs, it did not hinder the adsorption of iron on the calcium phosphate precipitate which forms in culture solutions containing calcium at alkaline reactions. By omitting calcium the adsorption of the iron was prevented, while the addition of sodium citrate to the medium served to maintain the iron in solution in cultures as alkaline as P_H 7.5. In such a modified culture solution maximum growth of *Chlorella*, as measured by the dry weight of the crop produced, appeared at P_H 7.5, the most alkaline solution of this series. The present paper presents the results of experiments with solutions of H-ion concentrations ranging from P_H 5.0 to 9.5, the purpose of the investigation being to establish if possible the alkaline limit for the growth of *Chlorella*.

Methods

The procedure adopted was the same as that previously reported. The culture medium consisted of equal portions of two solutions,

¹ Continuation of cooperative experiments begun at Cornell University under fellowships in the Biological Sciences, National Research Council. The writers wish to express their appreciation to both of these institutions for facilities which made the investigation possible.

designated Solution A and Solution B, which were combined after sterilization. Solution A contained 1.0 gm. NH_4NO_3 , 0.4 gm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 gm. sodium citrate, 0.8 mg. ferric iron, and 20 gm. glucose per liter. The iron was supplied from a standard solution of iron wire in dilute hydrochloric acid. Solution A was introduced in 25 cc. quantities into a number of Pyrex Erlenmeyer flasks, and these were immediately sterilized. Solution B consisted of eleven different phosphate buffer mixtures prepared from M/7.5 concentrations of K_2HPO_4 , KH_2PO_4 , and KOH, the mixtures being selected to give H-ion concentrations ranging from P_{H} 5.0 to 9.5. Since earlier experiments had established rather definitely the shape of the P_{H} growth curve on the acid side of P_{H} 5.0, solutions more acid than this were omitted from the present series of cultures. The buffer mixtures were prepared in lots of 100 cc. each and then introduced into Pyrex Erlenmeyer flasks in 25 cc. quantities, so that four replications of each P_{H} were provided. After sterilization the buffer mixtures were combined with Solution A. It should be noted that by the combination of Solutions A and B the concentrations recorded for these two solutions were reduced one-half in the final culture medium. Of the four replicates of each P_{H} , three were inoculated, the remaining one being used for determinations of the initial P_{H} and initial iron content. GILLESPIE's colorimetric method was used for all the H-ion concentration determinations, while the iron content was determined by the method of MARRIOTT and WOLF as modified by the authors (1). The inoculations were made from a uniform suspension of *Chlorella* sp. cells in 0.6 per cent NaCl, 0.5 cc. being used for each culture. The cells were obtained from a pure culture of the organism on agar slants. The usual aseptic precautions were observed throughout.

First experiment

After an interval of two weeks, during which the cultures were maintained in the greenhouse under partial shade, determinations were made of the dry weights of the crops produced and of the final H-ion concentrations of the culture solutions. Tests for soluble iron were also made on some of these solutions. The clear culture solution was obtained for these tests by centrifuging the contents of each

culture flask and decanting the supernatant liquid into large Pyrex test tubes. The algal cells were then washed into Gooch crucibles containing asbestos mats, and the dry weight of each crop ascertained after drying at 100° C. for 18 hours. The data obtained from the first experiment are presented in table I.

The tests for initial iron content showed as much soluble iron present at P_H 8.5 as at P_H 5.1, while at P_H 9.4 the amount appeared to fall slightly below that of the other solutions tested. The actual

TABLE I
DATA FROM FIRST EXPERIMENT

CULTURE NO.	P_H INITIAL	INITIAL FE PER CULTURE (MG.)	P_H FINAL			FINAL FE PER CULTURE (MG.)	DRY WEIGHT OF CROP (MG.)			AVERAGE CROP (MG.)
			A	B	C		A	B	C	
I.....	5.1	0.0475	4.6	4.5	4.6	0.023	71.0	81.1	76.6	76.2 ± 1.97*
2.....	5.8	0	5.5	5.5	5.5	0	72.4	83.8	77.6	77.9 ± 2.20
3.....	6.4	0	6.1	6.0	6.2	0	71.4	88.5	79.8	79.9 ± 2.34
4.....	6.9	0	6.6	6.7	†	0	77.9	81.8	†	79.8 ± 1.31
5.....	7.2	0	7.2	7.2	7.2	0	60.1	55.8	65.4	66.4 ± 1.87
6.....	7.6	0.0475	7.3	7.4	7.4	0.032	58.9	48.7	58.6	55.4 ± 2.26
7.....	7.8	0	7.6	7.6	7.5	0.046	29.0	42.3	46.9	39.4 ± 3.61
8.....	8.0	0.0475	7.7	8.0	7.9	0.032	18.1	04.6	07.4	10.0 ± 2.73
9.....	8.5	0.0475	0	0	0	0.037	0	0	0	0
10.....	8.9	0	0	0	0	0.037	0	0	0	0
II.....	9.4	0.0375	0	0	0	0.037	0	0	0	0

0, no determination made.

* Probable error determined by BESSEL'S formula.

† Culture contaminated.

amount of iron added to the original solution was equivalent to 0.02 mg. ferric iron per culture, the excess of nearly 0.03 mg. per culture found being accounted for as impurities in the reagents, most of it probably being derived from the glucose.

Final P_H determinations showed a slight increase in the H-ion concentrations, especially in the cultures in the acid end of the series. This was probably due, as we have shown elsewhere, to an unequal absorption of the ions of NH_4NO_3 . The final iron tests revealed a marked disappearance of soluble iron in the most acid culture where considerable growth had occurred. In the most alkaline cultures, where no trace of the alga was observed, there was still considerable iron in solution, although apparently some had been lost, probably by precipitation.

The dry weights of the crops are plotted in fig. 1. Growth was fairly uniform at H-ion concentrations from P_H 5.1 to 6.9, represented on the graph by an approximately straight line. From P_H 6.9 to 8.0 the growth declined rapidly, as indicated by the steep pitch of this part of the curve. In solutions more alkaline than P_H 8.0 no trace of growth was perceptible, the alkaline limit therefore being approximately 8.3. The decrease in the rate of growth in the

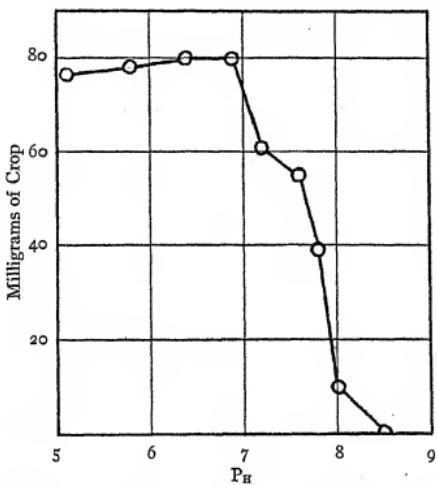


FIG. 1.—Growth- P_H curve, Experiment 1; alkaline limit for growth of *Chlorella*

alkaline cultures of this series cannot be attributed to unavailability of iron, as quantitative tests on these solutions showed that the bulk of the original iron was still present in soluble form.

Aside from the alkaline limit for growth as established by this curve, another feature of interest is the complete absence of any sharp maximum. There is some indication that growth is better at P_H 6.5 than at P_H 5.0 or 5.5, but the differences are not significant. This flat part of the curve corresponds closely to the portion of the curve between P_H 5.0 and 7.0 in the experiment previously reported (2, fig. 5).

Second experiment

The first experiment was repeated, using exactly the same solutions and procedure. The initial P_H determinations showed that the values of the first experiment had been very nearly duplicated, the only wide discrepancies being in the cultures more alkaline than P_H 8.2, where somewhat higher P_H values were recorded than in the previous cases. This can be accounted for by the interval of time

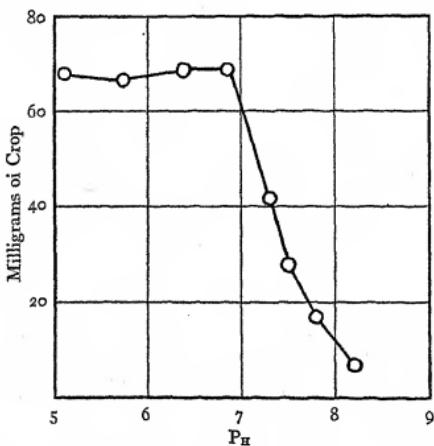


FIG. 2.—Growth- P_H curve, Experiment 2; alkaline limit for growth of *Chlorella*

elapsing between mixing the solutions and making the P_H determinations in the two cases. In the first experiment this time amounted to about 48 hours, during which period the most alkaline cultures no doubt absorbed sufficient CO_2 from the air to reduce the P_H values somewhat. In the second experiment the P_H determinations were made within 4 hours after mixing the solutions, which precluded much change in reaction due to the absorption of CO_2 . These cultures are beyond the limit of growth, however, so that the discrepancies do not affect the results obtained in the earlier experiment.

The detailed data obtained in the second experiment are presented in table II, and the dry weights of the crops are plotted in

fig. 2. The final P_h tests on the culture solutions showed, as in the previous experiment, that the H-ion concentrations of the solutions had increased somewhat during growth of the organism, especially in the acid end of the P_h range. Qualitative tests demonstrated the presence of considerable soluble iron in all of the culture solutions. The P_h growth curve is essentially like that of the first experiment, the point of decline being the same in both cases, as well as the limit for growth. The slope of the curve in the alkaline end of the series in the second experiment appears to be somewhat less steep than in

TABLE II
DATA FROM SECOND EXPERIMENT

CULTURE NO.	P_h INITIAL	INITIAL FE PER CULTURE (MG.)	P _h FINAL			FINAL FE (QUALITATIVE TEST)	DRY WEIGHT OF CROP (MG.)			AVERAGE CROP (MG.)
			A	B	C		A	B	C	
1.....	5.1	0.055	4.6	4.7	4.5	+	63.2	69.8	71.0	68.0
2.....	5.75	0	5.5	5.5	5.5	++	63.3	67.7	69.3	66.8
3.....	6.4	0	6.1	6.1	6.1	++	66.4	70.1	69.9	68.8
4.....	6.85	0	6.6	6.7	6.7	++	66.2	70.2	69.9	68.8
5.....	7.3	0	7.3	7.3	7.3	++	38.6	41.2	45.8	41.9
6.....	7.5	0.055	7.6	7.5	7.5	++	25.4	30.1	28.6	28.0
7.....	7.8	0	7.8	7.8	7.8	++	20.5	16.4	14.2	17.0
8.....	8.2	0.055	8.2	8.1	8.1	++	7.2	7.2	6.5	7.0
9.....	9.3	0	0	0	0	++	0
10.....	>9.75	0	0	0	0	0	0	0	0	0
11.....	>9.75	0.050	0	0	0	0	0	0	0	0

> = P_h greater than 9.75 (alkaline limit of Thymol Blue).

the previous series. The absence of a sharp maximum for growth is again clearly evident, as well as the absence of any indication of a double maximum characteristic of some cases of P_h growth curves.

Discussion

In the final experiment (Experiment 6) of our earlier paper and in the two experiments reported here, it was conclusively demonstrated that soluble iron was present and presumably available in all of the culture solutions regardless of the P_h . If the data from these three experiments are plotted on the same scale, the graph shown in fig. 3 is obtained. In this figure the maximum growth obtained in each experiment was taken as 100 and the rest of the data plotted accordingly. One exception to this procedure was made in the case

of Experiment 6 of our first paper, the three points representing growth in the most alkaline cultures of this series being left out of consideration. This curve gives an interesting picture of the relation of H-ion concentration to the growth of *Chlorella* sp.

The acid limit for growth as previously reported is close to P_H 3.4. This has been demonstrated in a number of experiments. The alka-

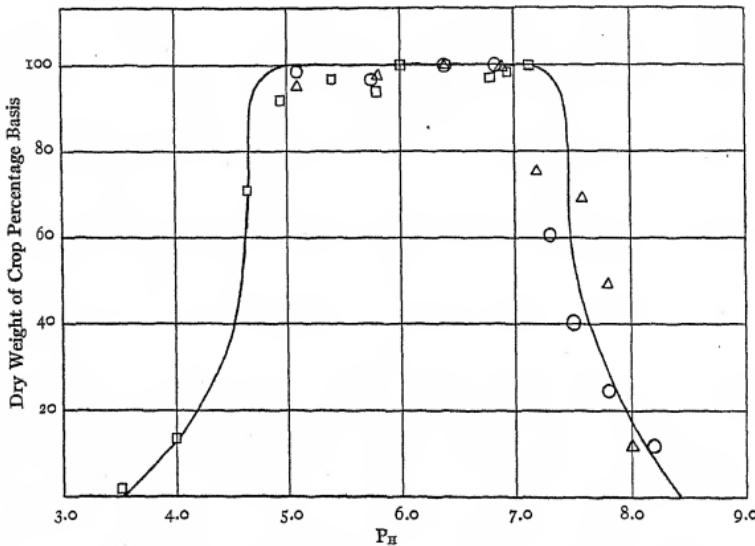


FIG. 3.—Composite growth- P_H curve prepared from data of three separate experiments, showing entire relation of P_H to growth of *Chlorella*: circles, Experiment 1; triangles, Experiment 2; squares, Experiment 6 of previous paper (2).

line limit for growth is about P_H 8.4 according to the data presented in this paper. There appears to be no definite high point or maximum; instead the curve ascends rapidly from both the acid and alkaline limits to a region lying between a P_H of 4.6 to a P_H of 7.0, in which the rate of growth is quite uniform.

Two suggestions might be offered to explain the "flat" portion of this curve. First, that the organism is not very sensitive to changes in H-ion concentration within this range, and second, that under the conditions of the experiment some other factor besides

H-ion or OH-ion concentration is limiting between P_h 4.6 and 7.0. At either end of the "flat" portion of the curve, however, a very slight difference in H-ion concentration on the one hand or in OH-ion concentration on the other will have a marked influence, since the curve drops off so steeply to the limits for growth on either side. As regards the nature of the factor or factors which may be limiting for growth in the region between P_h 4.6 and 7.0, it might be pertinent to mention certain results of investigations soon to be published by the writers on the iron requirement for *Chlorella*. It was found in these studies that, other conditions being the same, at H-ion concentrations near P_h 7.0, rather large amounts of iron were necessary for maximum growth, amounts much greater than used in the experiments reported in this paper. It is possible, therefore, that by increasing the iron content in this region the flat part of the curve would disappear.

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NEW SPECIES AND VARIETIES OF CHLOROPHYCEAE^{*}

L. H. TIFFANY

(WITH PLATE IX)

During the spring and early summer of 1926, the writer made numerous collections of algae along the Wabash River in southeastern Illinois and southwestern Indiana, which contained some species and varieties of *Oedogonium* and a species of *Spirogyra* apparently not previously described. The summer before Professor G. E. NICHOLS of Yale University had sent me a collection of algae from the Michigan Biological Station, Cheboygan, in which appeared an undescribed *Oedogonium*. This latter material was collected by Miss ALMA ACKLEY in the vicinity of Douglas Lake, Michigan, in August 1924. The following is a description of each of these forms, together with notes on distinguishing characteristics, relationship to other species, and time and place of collection. A Latin as well as an English diagnosis is given.

Spirogyra wabashensis, nov. sp.

Cellulis vegetativis $40-50 \mu \times 120-400 \mu$, dissepimentis planis; chromatophoris 2-4, anfractibus arctis 0.5-4.5; cellulis fructiferis, singulis vel binis inter cellulas vegetativas distributis, inflatis; tubo conjugationis ex cellula mascula emiso; zygosporis ellipsoideis, $56-76 \mu \times 110-150 \mu$, membrana media reticulata, lutea.

Vegetative cells $40-50 \mu \times 120-400 \mu$, with plane end walls, chromatophores 2-4, making 0.5-4.5 turns; fruiting cells inflated, single or in groups of two alternating with vegetative cells; conjugating tube formed by the male cell; zygosporule ellipsoid, $56-76 \mu \times 110-150 \mu$, median spore wall reticulate, yellow.

In the genus *Spirogyra* there have been described to date seven species and one variety in which the conjugating tube is formed wholly or nearly so by male cells. These are *S. punctata* Cleve, *S. punctata* Cleve var. *major* Hirn, *S. punctiformis* Transeau, *S. reflexa* Transeau, *S. micropunctata* Transeau, *S. rug-*

* Paper no. 173 from the Department of Botany, Ohio State University.

Ilosa Iwanof, *S. hydrodictya* Transeau, and *S. conspicua* Gay; *S. wabashensis* makes the eighth. The new species is distinguished from all these by its dimensions, number of chromatophores, and its beautifully reticulated median zygospore wall. One particularly noticeable feature of the species is the quite regular alternation of fruiting and vegetative cells, instead of the more usual series arrangement in conjugation. The chloroplasts in the sterile cells of the conjugating filaments are often much distorted and disintegrated, thus giving the appearance of a single chloroplast, or a chloroplast occupying a small portion of the cell length. The species was collected in a pool along the Big Four Railway, 3.5 miles south of Brownsville, Illinois, on May 14 and 20, 1926. Type in herb. L. H. T. collections nos. 21 and 42 (Illinois).—Fig. 1.

Oedogonium wabashense, nov. sp.

O. diocium, nannandrium, gynandrosporum; oogoniis singulis vel 2-3 continuis, ellipsoideis vel ovoideis, saepe terminalis, operculo apertis, circumscissione suprema, operculo saepe deciduo; oosporis eadem forma ac oogoniis (rarius globosis), complementibus vel non complementibus, membrana laevi; androsporangiis 1-3 cellularibus, subepigynis; cellulis vegetativis evidenter capitellatis; cellula fili basali forma, ut vulgo, elongata; nannandribus in oogoniis sedentibus; antheridio 1-? cellulari; crass. cell. veg. 12-20 μ , long. 36-64 μ ; crass. oogon. 36-42 μ , long. 44-60 μ ; crass. oospor. 34-38 μ , long. 40-55 μ ; crass. cell. andros. 12-16 μ , long. 10-20 μ ; crass. stip. nanndr. 12-16 μ , long. 24-40 μ ; crass. cell. antherid. 7-12 μ , long. 6-10 μ ; crass. cell. basal. 18-20 μ , long. 40-64 μ .

O. dioecious, nannandrous, gynandrosporous; oogonia single or in groups of 2-3, ellipsoid or ovoid, often terminal, operculate, division at the upper extremity of the oogonium, lid often deciduous; oospores of the same form as the oogonia (rarely globose), which they completely fill or not, walls smooth; androsporangia 1-3-celled, subepigynous; vegetative cells evidently capitellate; basal cells elongate; dwarf males on the oogonia; antheridia 1-?-celled; vegetative cells 12-20 $\mu \times$ 36-64 μ ; oogonia 36-42 $\mu \times$ 44-60 μ ; oospores 34-38 $\mu \times$ 40-55 μ ; androsporangial cells 12-16 $\mu \times$ 10-20 μ ; dwarf male stipes 12-16 $\mu \times$ 24-40 μ ; antheridial cells 7-12 $\mu \times$ 6-10 μ ; basal cells 18-20 $\mu \times$ 40-64 μ .

This species evidently belongs in the group with *O. obturcatum* Wittrock, *O. praticolum* Transeau, and *O. supremum* Tiffany. It differs from the first in having smaller fruiting cells and pluricellular dwarf males; from the second and

third it is distinguished by its gynandrosporous habit and smaller dimensions throughout. The lid of the non-terminal oogonium sometimes is much distorted, and gives one the impression of a superior division. When in this condition it has the general appearance of *O. ciliatum* (Hass.) Pringsh., differing however in size, shape of fruiting cells, absence of a terminal seta, and in being capitellate. It was collected in the bed of the old River de Shea near the Wabash River, 15 miles south of Vincennes, Indiana, May 26, 1926. Type in herb. L. H. T. collections nos. 47, 50, 52, 53 (Illinois).—Figs. 3, 4, 5.

OEDOGONIUM HOWARDII West, var. *minor*, nov. var.

Var. *omnibus partibus gracilioribus*: crass. cell. veg. $7-9 \mu$, long. $27-48 \mu$; crass. oogon. $26-29 \mu$, long. $28-36 \mu$; crass. oospor. $24-26 \mu$, long. $24-26 \mu$; crass. cell. antherid. $7-8 \mu$, long. $10-12 \mu$; crass. cell. basal. $12-14 \mu$, long. $10-12 \mu$.

Smaller than the type; vegetative cells $7-9 \mu \times 27-48 \mu$; oogonia $26-29 \mu \times 28-36 \mu$; oospores $24-26 \mu \times 24-26 \mu$; antheridial cells $7-8 \mu \times 10-12 \mu$; basal cells $12-14 \mu \times 10-12 \mu$.

The variety is distinguished from the type only by its smaller dimensions. The occasional subspherical basal cells and the usual shape of the oogonium, together with its much smaller size throughout, make it easily separable from *O. latiusculum* Tiffany. It was collected in a gravel pit pond 3 miles east of Pinkstaff, Illinois, May 28, 1926. Type in herb. L. H. T. collections nos. 55 and 56 (Illinois).—Figs. 6 and 7.

OEDOGONIUM BRAUNII Kuetz.; Pringsh. var. *Zehneri*, nov. var.

Var. *omnibus partibus crassioribus*, *oosporis ovoideis*; crass. cell. veg. $12-24 \mu$, long. $34-72 \mu$; crass. cell. suffult. $21-32 \mu$, long. $48-52 \mu$; crass. oogon. $40-50 \mu$, long. $48-60 \mu$; crass. oospor. $34-44 \mu$, long. $36-50 \mu$; crass. stip. nanndr. $8-10 \mu$, long. $16-24 \mu$; crass. cell. antherid. $7-8 \mu$, long. $8-12 \mu$.

Somewhat larger than the type in all dimensions; vegetative cells $12-24 \mu \times 34-72 \mu$; suffultory cells $21-32 \mu \times 48-52 \mu$; oogonia $40-50 \mu \times 48-60 \mu$; oospores $34-44 \mu \times 36-50 \mu$; dwarf male stipes $8-10 \mu \times 16-24 \mu$; antheridial cells $7-8 \mu \times 8-12 \mu$.

The habit of this variety is clearly that of *O. Braunii* Kuetzing; Pringsheim, differing in its larger size, particularly of the fruiting cells, and its differently shaped oospores. The dwarf male stipe is sometimes 4-5-celled and much elongated, having somewhat the appearance of another species of *Oedogonium* epiphytic upon it. In dimensions it is near *O. gallicum* Hirn, but the latter does not have swollen suffultory cells. It is larger than *O. Braunii* var. *hafniense*

(Hallas) Hirn. Collected from a pool along the Chicago and Eastern Illinois Railway, 3 miles south of Vincennes, Indiana, May 26, 1926. Type in herb. L. H. T. collections no. 44.—Fig. 2.

OEDOGONIUM ARESCHOUGII Wittr. var. *americanum*, nov. var.

Var. *idioandrospora*, oogoniis minoribus, androsporangii ad 11-cellularibus; crass. cell. veg. 8–12 μ , long. 40–80 μ ; crass. oogon. 29–36 μ , long. 26–40 μ ; crass. oospor. 23–26 μ , long. 22–26 μ ; crass. cell. andros. 7–9 μ , long. 7–11 μ .

Idioandrosporous, oogonia smaller, androsporangia to 11-celled; vegetative cells 8–12 $\mu \times$ 40–80 μ ; oogonia 29–36 $\mu \times$ 26–40 μ ; oospores 23–26 $\mu \times$ 22–26 μ ; androsporangial cells 7–9 $\mu \times$ 7–11 μ .

This form clearly belongs in the "Areschougii group," but its dimensions and idioandrosporous habit separate it from the type and from *f. robustum* Hirn. It was collected from a small lake near De Soto, Illinois, May 15, 1926. Type in herb. L. H. T. collections nos. 27, 28, and 29 (Illinois).—Figs. 8 and 9.

Oedogonium michiganense, nov. sp.

O. dioicum, nannandrium, gynandrosporum; oogoniis singulis vel 2–7 continuis, globosis vel ellipsoideo-globosis (rarius subglobosis), operculo apertis, circumscissione supra; oosporis globosis, oogoniis complementibus vel non complementibus, membrana triplici: episporio (in latere exteriore) laevi, mesosporio longitudinaliter costato (in sectione optica transversali undulato), costis crenulatis, interdum anastomosantibus, in medio oosporae c:a 12–24, endosporio laevi; cellulis suffultiis tumidis; cellulis vegetativis leviter capitellatis; cellula fili basali forma, ut vulgo, elongata; cellula terminali, quae interdum est oogonium, apice obtusa; nannandribus paullum curvatis in cellulis suffultiis sedentibus, antheridio interiore; crass. cell. veg. 12–24 μ , long. 80–160 μ ; crass. cell. suffult. 32–48 μ , long. 64–80 μ ; crass. oogon. 50–64 μ , long. 50–80 μ ; crass. oospor. 44–60 μ , long. 44–60 μ ; crass. cell. androsp. 16–20 μ , long. 16–20 μ ; crass. nannandr. 14–20 μ , long. 40–56 μ ; crass. cell. basal. 18–20 μ , long. 70–100 μ .

O. dioecious, nannandrous, gynandrosporous; oogonia single or in groups of 2–7, globose to ellipsoid-globose (rarely subglobose), operculate, division at the upper extremity of the oogonium; oospore globose, filling the oogonium or not, outer spore wall smooth, middle

wall with 12-24 crenulate, sometimes anastomosing, longitudinal ribs, inner wall smooth; suffultory cells enlarged; vegetative cells broadly capitellate; basal cells elongate; terminal cells, occasionally oogonia, apically obtuse; dwarf males, a little curved, on suffultory cells, antheridia interior; vegetative cells $12-24 \mu \times 80-160 \mu$; suffultory cells $32-48 \mu \times 64-80 \mu$; oogonia $50-64 \mu \times 50-80 \mu$; oospores $44-60 \mu \times 44-60 \mu$; androsporangial cells $16-20 \mu \times 16-20 \mu$; dwarf males $14-20 \mu \times 40-56 \mu$; basal cells $18-20 \mu \times 70-100 \mu$.

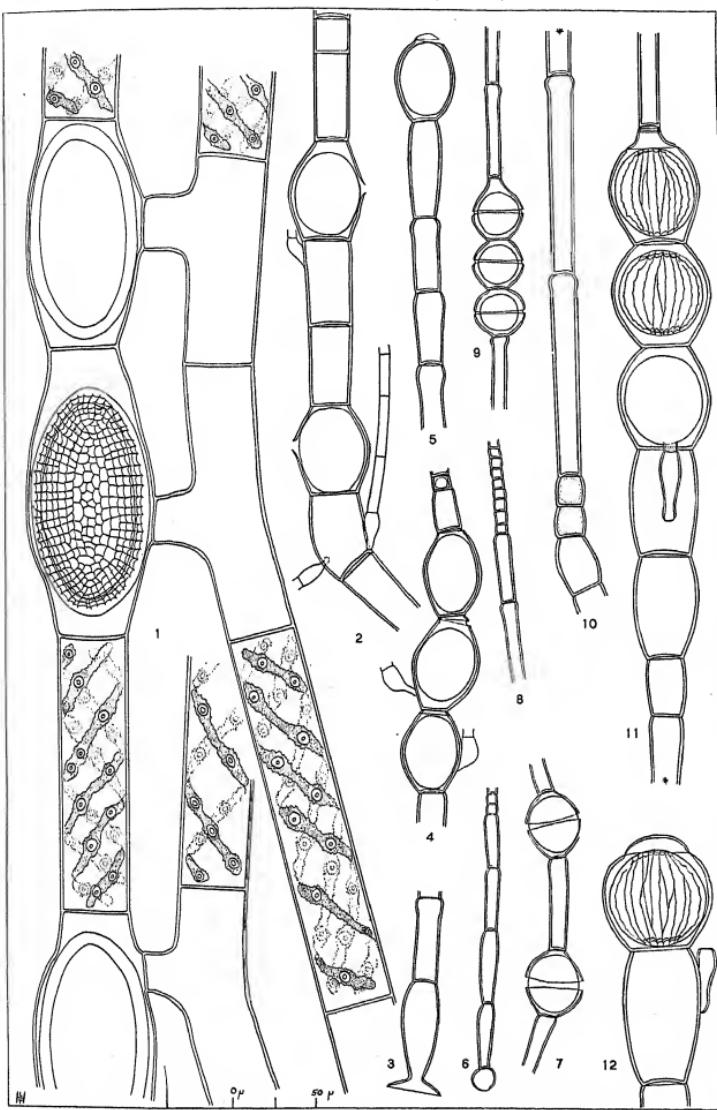
The position of the operculum, the markings of the oospore, the enlarged suffultory cells, and the capitellate vegetative cells present a combination of characteristics that necessitates a new species among the operculate forms of the genus. It was collected by Miss ALMA ACKLEY in small pools of Nichols' Bog and Smith's Bog in the vicinity of Douglas Lake, Cheboygan County, Michigan, during August 1924. Type in Nichols' collections nos. 202 and 212 and in L. H. T. Michigan collections nos. 1-6.—Figs. 10, 11, 12.

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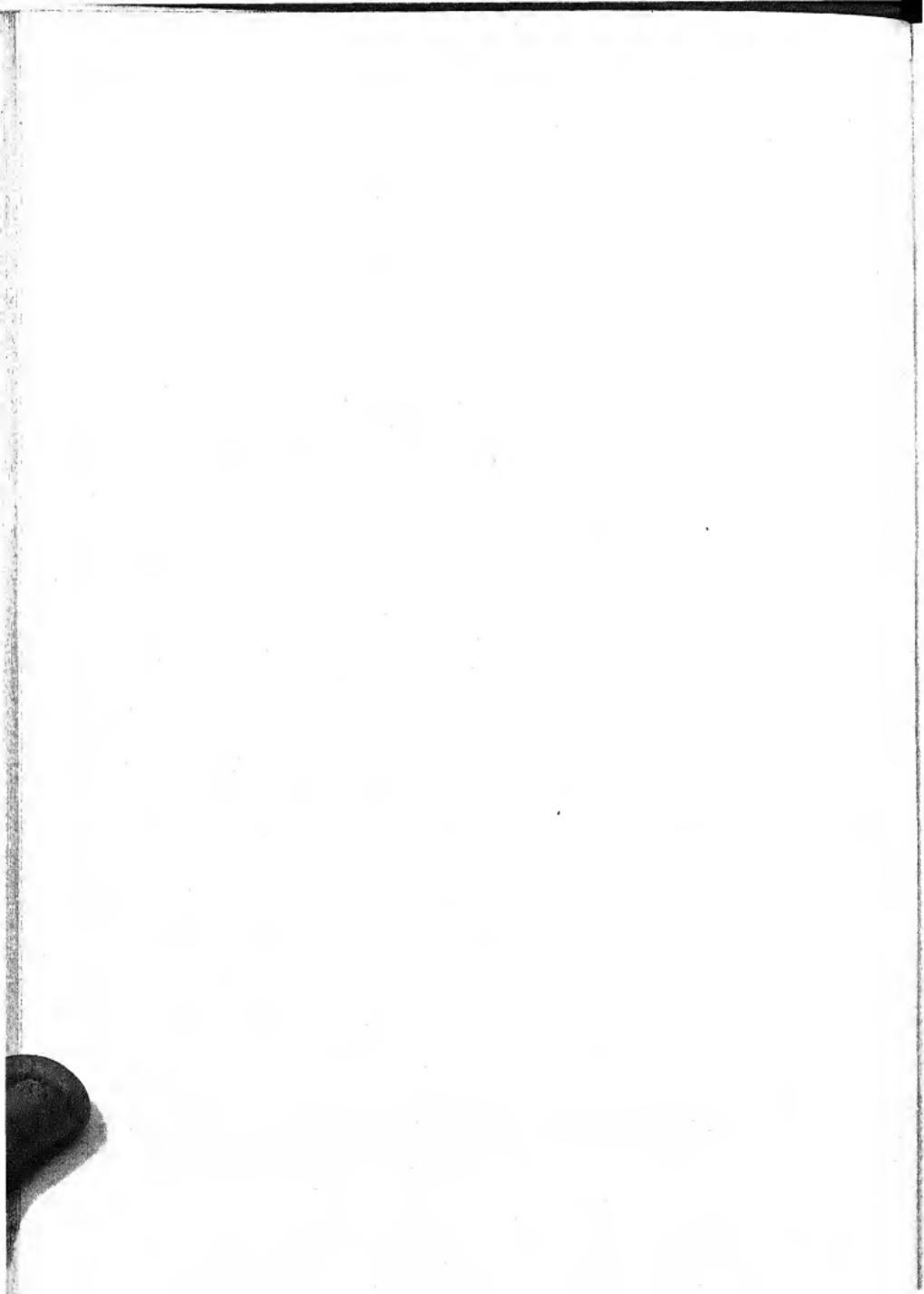
[Accepted for publication October 12, 1926]

EXPLANATION OF PLATE IX

- FIG. 1.—*Spirogyra wabashensis*, nov. sp.
FIG. 2.—*Oedogonium Braunii* Kuetz.; Pringsh. var. *Zehneri*, nov. var.
FIGS. 3-5.—*Oedogonium wabashense*, nov. sp.
FIGS. 6, 7.—*Oedogonium Howardii* West var. *minor*, nov. var.
FIGS. 8, 9.—*Oedogonium Arschouguii* Wittr. var. *americanum*, nov. var.
FIGS. 10-12.—*Oedogonium michiganense*, nov. sp.



TIFFANY on CHLOROPHYCEAE



EFFECT OF REACTION OF SOLUTION ON GROWTH OF ALFALFA¹

A. R. C. HAAS

(WITH THREE FIGURES)

Although an extensive literature is accumulating in regard to the effect of reaction on the growth of alfalfa, there is only partial agreement between the results of several recent investigations (1, 3, 4, 5). Some have found slightly alkaline solutions favorable to the growth of alfalfa, while others have found that approximately neutral or slightly alkaline culture solutions may have an injurious effect. Under field conditions alfalfa seems to do very well on soils which are slightly to moderately alkaline. It is therefore inconsistent with observations in the field that alfalfa should be injured by a neutral or slightly alkaline medium, or else our knowledge of what constitutes the actual reaction of the soil solution is seriously at fault. Lack of agreement between the results of culture solution tests and those of field trials may in part be due to the type of cultures and culture solutions employed. Solution cultures are ordinarily considered to be more reliable than sand or soil cultures in regard to the maintenance of the desired reaction. This may or may not be true, depending considerably upon the type of solution employed, the nature of the acid or alkali used in maintaining a given reaction, the frequency of renewal of the culture solution and of the desired P_H , as well as many other factors. By the use of controlled solution cultures, the writer has obtained results on the effect of reaction on the growth of alfalfa which are in harmony with those obtained on soils in the field, where the actual reaction is considered to be slightly to moderately alkaline.

The solution employed was a modified Hoagland solution in which double the usual amount of calcium nitrate was used and to which was added a trace of many ions not commonly used in solution cultures. Such a solution gives very favorable growth when

¹ Paper no. 154, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

used with solution cultures of citrus. It has the following composition expressed as parts per million:

NA	K	CA	Mg	NO ₃	CL	SO ₄	PO ₄
7	185	318	54	1214	10	216	105

with traces of ferric tartrate, aluminum sulphate, potassium iodide, titanium sulphate, potassium bromide, strontium nitrate, lithium nitrate, manganese sulphate, boric acid, and ammonium nitrate (2).

TABLE I
GROWTH OF ALFALFA IN MODIFIED HOAGLAND'S
SOLUTION; FIRST EXPERIMENT
TOTAL FRESH WEIGHT (GM.) OF ENTIRE PLANTS
IN EACH SERIES

SERIES	P _H 5	P _H 6	P _H 7	P _H 8
NaOH.....	8.0	9.0	12	11.5
Ca(OH) ₂	4.5	7.5	8	9.5

TABLE II
GROWTH OF ALFALFA IN MODIFIED HOAGLAND'S SOLUTION;
SECOND EXPERIMENT
TOTAL FRESH WEIGHT (GM.) OF ENTIRE PLANTS
IN EACH SERIES

SERIES	NO. OF PLANTS	P _H 5	P _H 6	P _H 7	P _H 8
NaOH.....	65	17.5	14.5	21	26
KOH.....	65	14.0	16.5	18	21

Distilled water containing small amounts of ferric tartrate was added to each culture every day. The solution had a P_H of 5.0-5.2, and was contained in enameled pails of 9 liter capacity, with covers of heavily tinned iron.

The alfalfa seeds were germinated in sand to which was added the modified Hoagland's solution, and were grown in the sand until the first leaves above the cotyledonary ones had developed. The seedlings were then washed free from most of the adhering sand, inserted

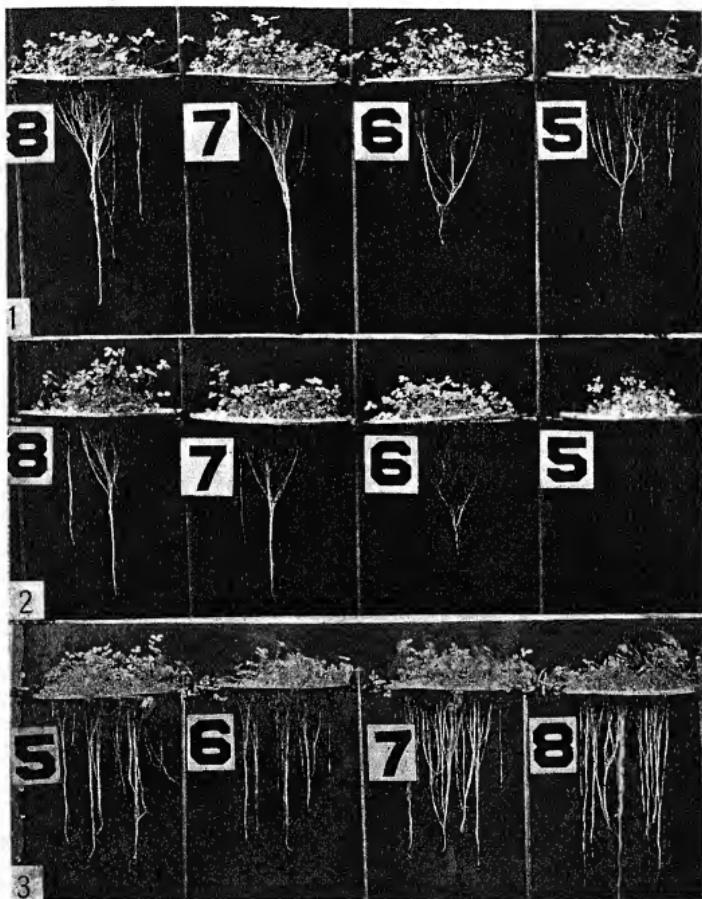


FIG. 1.—Growth of alfalfa in culture solutions maintained at various P_H values by addition of $NaOH$.

FIG. 2.—Growth of alfalfa in culture solutions maintained at various P_H values by addition of $Ca(OH)_2$.

FIG. 3—Growth of alfalfa in culture solutions maintained at various P_H values by addition of KOH.

into small holes in the covers, and left to support themselves without the use of plugs. The P_H value of the solution in the pails in each series was brought to 5, 6, 7, or 8 by additions of alkali two or three times daily as required. The culture solutions were renewed every 10 to 14 days.

FIRST EXPERIMENT.—On April 8, thirty-six alfalfa seedlings were placed in each solution culture and the P_H was brought to 5, 6, 7, and 8 by means of NaOH in one series and by means of $Ca(OH)_2$ in a second series. The cultures were continued until May 6; during the culture period the solutions were renewed three times. Table I gives the fresh weights of the plants in the first series, and figs. 1 and 2 show the growth in the two series. It is evident that in both series the growth was better at P_H 7 and 8 than at P_H 5 and 6.

SECOND EXPERIMENT.—On June 10 another series similar to the NaOH series was begun with alfalfa seedlings, but at higher temperatures because of the advancing summer. The plants were grown until June 29. On June 16 a series was begun in which KOH was employed in maintaining the desired reaction, and the cultures were continued until July 6. Fig. 3 shows the growth obtained, which is typical of both the series. The tops of the plants were 5–6 inches high but became wilted during the long photographic exposure in the hot glasshouse. Table II gives the fresh weights. As in the first experiment, growth was better in the neutral and alkaline solutions than in the acid ones.

Summary

Alfalfa seedlings were grown in Hoagland's solution modified by doubling the calcium nitrate, adding traces of certain other elements, and adjusting the P_H with NaOH, $Ca(OH)_2$, or KOH. Neither approximate neutrality nor moderate alkalinity appeared to be unfavorable to growth. The average growth was least at P_H 5 and greatest at about P_H 8.

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CURRENT LITERATURE

BOOK REVIEWS

Cytology of flowering plants

A comprehensive treatment of the cytology of gymnosperms and angiosperms has long been needed by investigators in this field. The *Morphology of angiosperms* by COULTER and CHAMBERLAIN treated the whole subject of morphology, but the date of its publication is 1903, whereas most of the critical cytological literature, and nearly all of the cytology of genetics has developed since that time. The third edition of the *Morphology of gymnosperms*, by the same authors, brings the literature up to 1917, but here again the treatment covers the whole field of morphology. Consequently, the recent book by SCHÜRHOFF¹ covers a little occupied field. It does not pretend to present the results of the author's own investigations, although these have been extensive; but is written in textbook style, and presents a critical and well organized summary of the literature up to 1926. The presentation is scholarly and unprejudiced, and work from other countries is given adequate recognition.

The first part, on general cytology, deals with rather strictly nuclear phenomena and gametophytes. It includes a discussion of the significance cytological features may have in systematic botany. In the second part the cytology of the various groups of seed plants is treated separately, with copious references to literature. In many families the chromosome numbers are given, and features which may be of value in any phylogenetic treatment of taxonomy are emphasized. It is well known that most cytological investigations have been made upon fixed and stained material. Studies on living material are also presented, but the author concludes that such studies have added little to our knowledge, except to confirm the results obtained by microtechnical methods.

The haploid generation has not been used much in the taxonomy of higher plants, and keys based upon cytological features would be hard to apply; but the comprehensive treatment of the haploid generation assembles and organizes the data so that they can be used in taxonomic problems, so far as they are concerned with phylogeny at least.

There are more than 2000 references to literature, and the volume undoubtedly will facilitate investigation in both the morphology and cytology of seed plants.—C. J. CHAMBERLAIN.

¹ SCHÜRHOFF, P. N., Die Zytologie der Blüthenpflanzen. 8vo. pp. xi+792. figs. 282. Stuttgart: Ferdinand Enke. 1926.

Fungi of Middle Europe

Number 1 and part of number 2 (the colored plates only) of Volume I of a new work on fungi² have made their appearance. This volume is to be devoted to the Boletaceae. This series of publications is under the auspices of the German Society of Mycology, the German Botanical Society, and the German Society for Teaching Biology, and is intended to cover the entire field of mycology, each group of fungi being assigned to a specialist for monographic presentation. So far as possible, the fungi are to be illustrated in natural colors, in natural size, and various species are to be represented by more than one specimen, so that both the range in form and color, as well as the various stages in the life history of the species will be given.

Number 1, which is a representative of the 20-25 numbers of Volume I, contains two colored plates, two plates in black and white, and four pages of text. The colored illustrations are beautifully done. The text consists of description of the plates, and in addition presents material under the following headings: original and modern detailed description and diagnosis of the species; economic and toxicological aspects; possible confusion with other species; microscopic studies; habitat and geographic distribution; history; bibliography.

The work is excellent and should prove exceedingly interesting and useful to American specialists and amateurs in the field of mycology. It is a matter of regret that a similar work on North American fungi is not under way.—G. K. K. LINK.

NOTES FOR STUDENTS

Suction force of plant cells.—In a lengthy paper BLUM³ continues the discussion of suction force of plant cells, using numerous alpine species. By means of URSPRUNG's simplified method he found very high suction forces, so that the direct readings of BERKELEY and HARTLEY, and of FRAZER and MYRIK were used for the determination of exact volumes. Aerial tissues were always found to have a higher suction force than root tissues. *Campanula glomerata* was found to have a suction force in its leaves of 44.5 atmospheres during the summer. The values varied with season, rainfall, and other variable factors, as would be expected. Floral parts also had high values in some cases; thus the petals of *Lotus corniculatus* showed 34.5 atmospheres, but *Pinguicula alpina* had a low value for its flowers, only 2 atmospheres. Of the factors modifying suction force of cells, soil moisture has the chief influence. When the soil is no longer very moist, atmospheric humidity changes modify the suction force rather strongly. With the diurnal changes in saturation deficit the suction force keeps

² Die Pilze Mitteleuropas, under the editorship of KNIEP, H. (Berlin), CLAUSSSEN, P. (Marburg), and BASZ, J. (Stuttgart). Leipzig: W. Klinghardt. Band I. no. 1. 1926. M. 4.00.

³ BLUM, G., Untersuchungen über die Saugkraft einiger Alpenpflanzen. Beih. Bot. Centralbl. 43:1-100. 1926.

pace in a general way; and dry and moist habitats always have high or low average suction force values.

As standards of reference the behavior of *Taraxacum* and *Bellis* was taken, and other plants compared with them. Thus the limits of variability of suction force of one species could be compared with another in the same and different habitats. Some exceptional plants were noted, such as *Ranunculus aconitifolius*, which occupied moister areas, yet always had very high suction values as compared with the standard plants. Also the xerophile *Thymus serpyllum*, occupying drier habitats, had consistently lower values than the standards. Ecological groups were compared, and lower average values were found for the moisture indicators, fertility indicators, and shade plants. Higher average values were found for the arid indicators, calcicoles, poor land indicators, and sun plants. These suction force studies show in a different way, by plasmolytic means, just what HARRIS' studies of freezing point depressions have shown, a general correspondence of plant cells to the conditions of the habitat.

The reviewer dislikes the term suction force. If we speak of suction at all, it is a suction tension, but it is questionable whether we should use the word suction at all. If a careful analysis of water movement is made, it will always be found that water is the active compound. The cell contents merely provide a medium of lower free water content into which water from regions of greater free water content moves. The pressure in any cell is caused by the free water entering from outside the cell. If the pressure in a street car, when overcrowded with people who try to enter after the car is full, can be called a suction pressure, then the pressures in a cell can be called suction pressure, and the force of entry called a suction tension. URSPRUNG and BLUM have shown how to measure osmotic pressure accurately by the plasmolytic method, a method which has been a very faulty tool until now, and this is an important contribution. The reviewer, however, sees no good reason for using any other term than osmotic pressure in connection with the turgidity of plant cells.—C. A. SHULL.

Function of iodine.—All organisms that inhabit the earth use iodine, according to STOKLASA.⁴ In forms like bacteria, that respire very rapidly, he believes iodine serves in the oxidase system. Among higher plants, halophytes, hygrophiles, and hydrophytes use the most iodine. Here too the iodine is thought to be related to the respiratory system. Root systems with iodine give off more CO₂ than without iodine. This result might be due to more vigorous growth; but STOKLASA points out that in the presence of iron and radium emanation there is much more CO₂ output in iodine-rich roots, than in those poor in iodine. Iodine in some way also prevents development of high acidity internally. *Senecio vulgaris*, without iodine added to the soil, showed a P_H of 2.68 and 3.02

⁴ STOKLASA, JULIUS, Die physiologische Funktion des Jods beim Bau- und Betriebsstoffwechsel in der chlorophyllhaltigen und chlorophyllosen Zelle. Biochem. Zeitschr. 176:38-61. 1926.

for aerial parts and roots respectively, while the specimens given iodine showed a P_H of 4.78 in the aerial portions, and 5.1 for the roots. This internal control of H-ion concentration prevents acid depression of enzyme activity, and injury to the chlorophyll apparatus. The chlorophylous parts are always richer in iodine than the root system. The transformations of energy and materials in general are accelerated in plants having iodine, the basal metabolism being favored by its presence. Since halophytes and other iodine users have more furfuroids in them, STOKLASA thinks the iodine favors the transformation of cane sugar into furfuroids. But this is really arguing that any differences found in plants that use iodine are due to the iodine, a loose type of reasoning of which we have far too much in biology.

Certain plants take up iodine and deposit it in organic form. These plants are referred to as iodine-saturated. The pharmacological usefulness of such plants is pointed out. Potatoes, radishes, and tomatoes can be saturated in this way if iodine fertilizer is added to the soil. Experimenting on himself by eating vegetables grown without iodine, and then with iodinized fruits and vegetables of the same kind, he shows that the acidity of urine is much decreased by an iodine-containing diet. However, inorganic iodine does not give good results. The iodine is needed in organic form. The proper way, then, to get our iodine, is not by using iodized salt, but by growing vegetables on soils furnished with something like 50 ppm of iodine, and using these for food. Whatever may be the needs of plants for iodine, and the exact functions of iodine in the plant body, the need of iodine by animals and man has been fully demonstrated, and the pathological effects of lack of iodine are in part known. STOKLASA's method of applying the dosage is certainly the proper method. Sea foods, and vegetables grown on iodine fertilized soils, would no doubt be valuable in the diet of everybody.—C. A. SHULL.

Chemical determination of sex.—A number of workers have been attracted by the claim of MANOILOFF that the sexes can be distinguished by means of a chemical reaction. Essentially his test is based on the idea that alcoholic extracts from female tissues possess a reducing substance, while those from males have an oxidizing effect. The color changes occurring in a mixture of papayotin, dahlia, potassium permanganate, hydrochloric acid, and thiosinamin, to which extract from males or females has been added, are believed to indicate with a high degree of accuracy whether the extracts come from one sex or the other.

The method has been subjected to a critical test by ALSTERBURG and HAKANSSON,⁵ who used not only rabbits, sparrows, and fish, but many kinds of plants, hermaphroditic, monoecious, and dioecious. *Magnolia*, *Tulipa*, and *Paeonia* were used as hermaphrodites, *Carpinus*, *Fagus*, and *Alnus* among the monoecious forms, and four species of dioecious willows, *Rhodeola rosea*, *Peta-*

⁵ ALSTERBURG, GUSTAF, and HAKANSSON, ARTHUR, Über Manoiloffs Reaktionen und die Möglichkeit, mit Hilfe dieser das Geschlecht zu bestimmen. Biochem. Zeitschr. 176:251-265. 1926.

sites albus, and *Melandrium rubrum* among the latter group. In more than 50 per cent of the cases the reaction failed to indicate the sex. This is a much poorer showing than is claimed by MANOLOFF himself, and by SATINA and coworkers in this country.

They conclude that the MANOLOFF reaction is only quantitative, not qualitative. They claim that there is no enzyme involved, and no sex hormone. The papayotin is held to be useless in the mixture, as the only feature actually involved is the ability of organic substances to reduce, to a greater or less degree, potassium permanganate. The same effects can be produced by purely inorganic substances, as by the MANOLOFF reaction. The dahlia or methyl violet may also have some reducing action, but mainly serve to show whether oxidation or reduction is dominant. If oxidation prevails, the mixture goes colorless. If reduction is greater, a part of the pigment remains to give color as found by MANOLOFF. The permanganate is an oxidizer, and by using a measured amount, one has a measure of the reducing substance in the extract. The rôle of thiosinamin is not very clear, but excess of oxidation medium may be taken care of by this means.

With organic material from many sources, the authors have been quite unable to substantiate MANOLOFF's claim for this method of determining the sex of individual animals or plants.—C. A. SHULL.

Endosperm activity.—Using sterile conditions, GRÜNFELD⁶ shows that corn endosperm freed of embryo and placed in 10 cc. of water will empty itself of reserves in 35–40 days at 25° C. As it takes a germinating embryo only half as long to do this, the embryo must play some part in the hydrolytic process. It probably excretes some enzyme, and at any rate keeps the products from accumulating and delaying the hydrolysis. In isolated endosperm the rate of hydrolysis rises for 5–7 days to a maximum, maintains this high rate for several days, then falls off during the next 10 days to a low value. Complete cessation of hydrolysis occurs when a sugar concentration of 4–5 per cent in the surrounding water is reached. The medium was found to contain mainly glucose, much maltose, some cane sugar, and traces of fructose, besides an undetermined carbohydrate.

Partly emptied endosperms will reverse the process and fill up with starch if given an aqueous solution mixture of 5 per cent dextrose, 5 per cent cane sugar, 5 per cent dextrine, and the natural hydrolytic products of endosperm hydrolysis. Without these products the reversal does not take place. From the ability to digest itself, and refill itself with starch, GRÜNFELD assumes that the endosperm is alive. It may be, but why should not a non-diffusing diastatic enzyme be able to do the same thing in cells that are not actually living?—C. A. SHULL.

⁶ GRÜNFELD, OTTO, Über die Entleerung und Wiederauffüllung isolierter Getreideendosperme, insbesondere von Mais, unter aseptischen Bedingungen. Beih. Bot. Centrallbl. 43:167–200. 1926.

THE
BOTANICAL GAZETTE

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GROUPING OF LEGUMES ACCORDING TO
BIOLOGICAL REACTIONS OF THEIR
SEED PROTEINS¹

POSSIBLE EXPLANATION OF PHENOMENON OF
CROSS INOCULATION

I. L. BALDWIN, E. B. FRED, AND E. G. HASTINGS
(WITH TWO FIGURES)

Introduction

Among the root nodule bacteria of leguminous plants a number of races have developed, each capable of causing the formation of nodules on the roots of certain species of the Leguminosae and not on others. The leguminous plants, on the basis of their relation to the root nodule bacteria, have been divided into "cross inoculation" groups, any member of which may be infected by the bacteria from any other member of the group.

The adaptations of the various races of the root nodule bacteria to the different species of the Leguminosae and the phenomenon of cross inoculation have occasioned speculation and study since 1891, when NOBBE and his co-workers (26) discovered that the organism of pea nodules is unable to form nodules on the roots of serratella and lupine.

Determination of these cross inoculation groups is of great practical importance in the culture of leguminous plants, and such studies have been made of practically all the cultivated legumes.

¹ Contribution from the department of agricultural bacteriology, University of Wisconsin.

On the other hand, the factors responsible for or controlling the adaptation of the various races of nodule bacteria to the specific host plants present a question of scientific interest which has not received careful study. While these groups of bacteria possess differences in their adaptations to the host plants, they are almost indistinguishable by the common bacteriological methods.

Generally speaking, the species belonging to any cross inoculation group represent those closely related botanically; however this is not true in all cases. In the group commonly spoken of as the cowpea group, there are at least 10 genera represented.

Why should the organism responsible for nodule formation on the roots of the lima bean be able to infect the roots of the cowpea and not those of the garden bean? The answer must be found in the differences among the various species of the host plant and the various races of the root nodule organism. And, inasmuch as the question is not one of the formation or lack of formation of root nodules on a particular plant species, but rather one of the adaptation of a certain bacterial race to the particular plant species, it would seem probable that the chemical phases of the problem are of more importance than the morphological ones.

A similar problem is presented in a study of the factors which render certain varieties of a plant species resistant to invasion by a plant parasite, while other varieties of the same species are susceptible to attack by the same parasite. Since this problem is one of the ability or lack of ability on the part of the plant to withstand the attack of the parasite, it is conceivable that either or both physiological and morphological factors in the plant may play a part. Investigations in this field indicate that in some cases the physiological conditions are the controlling factors, while in others morphological variations seem to determine the resistance or susceptibility to the attack of the parasite.

DICKSON and HOLBERT (9), working on the resistance of the corn plant to root and stalk rot, found that the resistance or susceptibility of the corn plant was connected with the rate and type of carbohydrate metabolism. NELSON (25), by serological methods, was able to distinguish between the proteins of a wilt-resistant flax and a non-resistant variety. WILLAMAN, PERVIER, and TRIEBOLD (41)

studied the resistance of certain varieties of plums to attack by *Sclerotinia cinerea*, and could find no significant differences between the physiology of the resistant and susceptible varieties, but did find differences in the physical character of the fruit which they believed to be responsible for the differences in the resistance of the host plant to invasion by the parasite. Many other similar examples might be mentioned.

An interesting parallelism to the grouping of the legumes by the root nodule organisms is furnished by the grouping of the legumes by many of the plant parasites. GARDNER (11) reported that *Bact. vignae* which causes the bacterial spot disease of cowpea also attacks catjang, adzuki bean, velvet bean, hyacinth bean, and tick trefoil, all of which belong in a single cross inoculation group.

Relatively few data are available to explain the adaptation of the root nodule bacteria to a particular plant species. SIMON (32) suggested that the protein characteristics of the plant may explain the adaptation of bacteria to the plant. No experimental data on this point are given, and a study of the later literature fails to reveal any further communications relative to the subject. FRED and DAVENPORT (10), in a study of the influence of reaction on the root nodule bacteria and by a comparison with the results of other workers upon the influence of reaction upon the leguminous plants, found a correlation between the two.

During the past two decades considerable effort has been expended in the determination of the so-called natural system of plant grouping or classification of serological methods. Inasmuch as the fragmentary work, which had been carried out with legumes, checks with the groupings established by cross inoculation, it was felt advisable to repeat and extend this work to include all the more commonly cultivated legumes.

Plant groups as determined by serological methods

In studying plant relationships at least five different serological reactions have been utilized: (1) precipitin, (2) complement fixation, (3) anaphylaxis, (4) conglutinin, and (5) Abderhalden. Of these, the precipitin reaction was the first to be used in the study of plant relationships, and has been more generally used than any of the others.

The complement fixation, anaphylaxis, and conglutinin reactions, however, have all proved valuable in this connection. The Abderhalden reaction has not been found suitable for this work.

The work with the higher plants may be divided into two groups. Certain workers have been interested in the possibility of differentiating between various plants, while others have attempted to bring together into groups closely related ones. The attempt to separate one plant from another by serological methods dates back to KOWARSKI (19), who in 1901 was able to distinguish between wheat, rye, oats, and peas by the precipitin reaction. Many workers have since found it possible to differentiate between species by the various serological reactions.

RELANDER (29, 30) was probably the first to demonstrate that the technique of the reactions can be so adjusted as to enable differences to be noted between varieties within a species. He was able to differentiate between American, Norwegian, Finnish, and Italian red clovers, and between 2-rowed and 6-rowed barley by the use of the precipitin test. BALLNER and BURROWS (4) demonstrated small differences between strains in both beans and peas by the use of the complement fixation reaction.

MAGNUS and FRIEDENTHAL probably were the first to use serological methods in a study of plant relationships. In their first work (22) they investigated the relationships between certain fungi. In a later paper (23) they reported the results of work with antigens secured from the higher plants. In this connection they stated: "The important discovery was made, that the duration of the immunization had an important influence not only quantitatively but also qualitatively, in that with longer immunization the degree of relationship always assumed wider limits." MAGNUS (21), working with members of the grass family, suggested that the best method of establishing relationships is progressively to immunize the animal and note the widening range of the precipitation. In support of this idea he gave the data tabulated on page 221.

Similar results were secured with other members of the grass family. However, in carrying his treatments as long as 245 days, in no case was a reaction secured with a non-grass.

MEZ and GOLKE (24) were the first to make a serious attempt to study plant relationships by means of serological studies. While the principal portion of their work was with the conglutinin method, they also used the precipitin reaction. They worked with a great number of angiosperms, and grouped them according to the relationships exhibited in these tests. Since that time MEZ and the workers in his laboratory have carried on considerable experimental work in their study of the so-called natural relationships of plants. Little of this work has touched upon legumes. RIVES (28) suggested the

TREATMENT OF ANIMAL (DAYS)	POSITIVE REACTION WITH	NEGATIVE REACTION WITH
	ANTIWHEAT SERUM	
19.....	Triticum, Hordeum, Secale	Zea
29.....	All the above plus Zea	Lolium, Bromus
36.....	All the above plus Lolium and Bromus	Oryza
146.....	All the above plus Oryza
<hr/>		
ANTIMAIZE SERUM		
7.....	Zea, Euchlaena	Panicum, Triticum, and Hordeum
28.....	All the above plus Panicum
50.....	All the above plus Triticum and Hordeum	Phalaris, Festuca, Bromus, and Lolium
109.....	All the above plus Phalaris, Festuca, Bromus, and Lolium	Avena
130.....	All the above plus Avena

use of the precipitin method to measure the relationships existing between grapes. He found that grape hybrids which showed close relationships with the precipitin reaction could be grafted upon each other. Those which did not show close relationships by the precipitin reaction could not be grafted together.

The early workers in this field used crude extracts of the plant material, usually physiological salt solution extracts. GASIS (12) and WELLS (33) were the first to use purified plant proteins in immunological work. GASIS used the precipitin reaction and was successful in separating peas and beans, as well as several other plants. WELLS used purified zein and gliadin and was able to demonstrate that the anaphylactic reaction was equally as specific

and as severe with vegetable antigens as with animal proteins. WELLS and his associates followed up his early work with considerable additional experimentation (20, 34-38, 40). This work was largely with the anaphylactic reaction, although the other reactions were also used. Carefully prepared and purified plant proteins were used in each case. The following facts of particular interest in this work were demonstrated. (1) The specificity of the biological reactions is dependent upon the chemical constitution of the antigen and not upon its origin. Thus the use of the biological method in the determination of plant relationships is a measure of protein similarity. (2) Natural sensitization of guinea pigs may occur by feeding of the antigen. (3) Animals may be sensitized to two or more proteins at the same time, and after intoxication with one of the proteins, providing death does not occur, a reaction may be obtained by intoxication with the other.

The results of the previous work with legumes are presented in table I. This table shows that all the work, extending over a considerable period, done with a variety of serological methods, and in most cases unhampered by the influence of previous results, is in substantial agreement. Practically all of the work indicates that pea, vetch, lentil, and horse bean are more or less closely related and form one group. Among the beans *Phaseolus vulgaris*, *P. multiflorus*, and *P. nanus* have been grouped together. *P. raditus*, however, has been shown to possess a different protein complex from that of *P. vulgaris*. Red and white clover have been grouped together, with alfalfa closely related but still separate. Seven species of *Acacia* have been grouped together and separated from pea, vetch, and bean. Soy bean has been separated from all others. Ground nut (*Aipos tuberosa*) has been shown to be different from either the pea or bean group. Lupine did not react with pea and lentil. Vignin, the purified globulin from cowpea, while reacting to a certain extent with the globulins of the other legumes, still appears to be different from any of the others used, that is, pea, vetch, lentil, soy bean, and horse bean.

While this work covers only a very small proportion of the legumes in which one is interested from the standpoint of cross

TABLE I
PREVIOUS WORK ON BIOLOGICAL SEPARATION OF LEGUMES

WORKER	DATE	REFER- ENCE	METHOD	RESULTS
Bertarelli.....	1903	5	Precipitin	Pea, bean, lentil, vetch, and horse bean, all separate
Magnus and Friedenthal.....	1907	23	Precipitin	Pea and vetch together; lupine separate group
Gasis.....	1908	12	Precipitin	Pea and lentil together; <i>Phaseolus vulgaris</i> and <i>P. multiflorus</i> nearly alike
Raubitschek.....	1909	27	Precipitin	Pea and lentil together; bean separate group
Inomata.....	1910	1, 2	Precipitin, anaphylaxis	Soy bean and horse bean different
Inomata.....	1910	14	Anaphylaxis	Different varieties of lentil alike
Ballner.....	1910	3	Complement fixation	Pea and lentil separate
Karavasa.....	1910	16	Anaphylaxis	Pea, lentil, and "grosse" bean different from bean
Chapman.....	1910	6	Precipitin	Seven strains of <i>Acacia</i> together
Relander.....	1911	30	Precipitin	Pea more closely related to <i>Acacia</i> than bean or vetch
Ballner and Burrows.....	1911	4	Complement fixation	Red clover and vetch different; separates strains of red clover
Wendelstadt and Feltner.....	1911	39	Precipitin, anaphylaxis, complement fixation	Pea, vetch, and lentil together
Sauli.....	1911	31	Conglutinin	<i>Phaseolus minus</i> and <i>P. multiflorus</i> together
Golle.....	1915	13	Conglutinin, precipitin	Ground nut different from
Koletsu.....	1917	18	Precipitin	White and turkish bean together } either
Kohiz.....	1923	17	Conglutinin, precipitin	Red and white clover together } Also separated strains in horse
Ishiwara.....	1923	15	Alderhalden	Alfalfa close to clovers but different }
Wells et al.*.....	1941	34	Anaphylaxis	All legumes together; pea and vetch nearest to lentil
	1913	35		All legumes together; none close to horse bean
	1914	36		All legumes together
	1915	37		Pea and soy bean separate
	1916	38		Soy bean separate from others
				Cowpea indistinct but separate
				Pea, vetch, lentil, and horse bean together
				Kidney bean and adzuki bean separate from each other

* Worked with purified proteins.

inoculation, the protein groups in every case agree with the established cross inoculation groups.

Experimentation

Previous work in this field has established the value of serological methods for classifying plant species into groups, whose members are more or less closely related physiologically; also that all groups which have been made of the Leguminosae by these methods agree with the cross inoculation groups of the root nodule bacteria. With these facts in mind, it was arranged to investigate the protein relationships existing between other members of the Leguminosae, including all the commonly cultivated legumes. The plants studied are grouped on the opposite page.

The seeds of the plants were selected for study, since practically all of the previous work had been done with seeds, and also since these represent the most readily available sources of material. Since the purpose was to establish relationships between species, rather than to detect differences between closely related forms, the methods of procedure were chosen with that in mind. Due both to their suitability and to their ease of manipulation, the precipitin and anaphylaxis reactions were chosen for this work. The use of the DALE (8) uterus strip method of anaphylaxis provides an additional advantage, in that a permanent visual record of the course of the reaction is secured.

Earlier workers with naturally occurring protein mixtures of either plant or animal origin, such as MAGNUS (21) and others, have shown that the length of duration of the immunization process influences to a considerable degree the breadth of the relationships exhibited by the antiserum. Serum produced by weak immunization and of low titre is of particular value in differentiating between closely allied forms (RELANDER 30). On the other hand, severe and long continued immunization tends to produce a serum reacting with a wide range of related species; GOHLKE (13) found all the legumes rather closely related. In this work it was attempted to secure moderate immunization with the production of sera which would be useful in the determination of plant relationships.

Since the purpose of this work was to study the physiological relationships, in so far as protein characteristics are concerned, it

was felt that an extract of the seeds which would contain some of all the proteins present would be more suitable for use as an antigen than a solution of the purified plant proteins. After a review of the

Alfalfa group	{	1. Alfalfa, <i>Medicago sativa</i>
		2. Hubam clover
		3. White sweet clover, <i>Melilotus alba</i>
		4. Fenugreek, <i>Trigonella foenum-graecum</i>
Clover	{	5. Red clover, <i>Trifolium pratense</i>
		6. White clover, <i>T. repens</i>
		7. Alsike clover, <i>T. hybridum</i>
Pea group	{	8. Garden pea, <i>Pisum sativum</i>
		9. Canada field pea, <i>P. sativum arvense</i>
		10. Broad or horse bean, <i>Vicia faba</i>
		11. Hairy vetch, <i>V. villosa</i>
		12. Spring vetch, <i>V. sativa</i>
		13. Wild vetch, <i>V. angustifolia</i>
Cowpea group	{	14. Cowpea, <i>Vigna sinensis</i>
		15. Lima bean, <i>Phaseolus limensis</i>
		16. Japan clover, <i>Lespedeza striata</i>
		17. Velvet bean, <i>Mucuna utilis</i>
		18. Beggarweed, <i>Desmodium tortuosum</i>
		19. Furze ?, <i>Ulex europaeus</i>
		20. Scotch broom ?, <i>Cytisus scoparius</i>
Garden bean group		21. Garden bean, <i>Phaseolus vulgaris</i>
Soy bean group		22. Soy bean, <i>Soja max</i>
Lupine group	{	23. Blue lupine, <i>Lupinus angustifolius</i>
		24. Yellow lupine, <i>L. luteus</i>
		25. White lupine, <i>L. alba</i>
		26. Serradella, <i>Ornithopus sativus</i>
Sanfoin group	{	27. Sanfoin, <i>Onobrychis sativa</i>
		28. Dalea, * <i>Dalea alopecuroides</i>
		29. Kidney vetch, * <i>Anthyllis vulneraria</i>

* Dalea and kidney vetch have not been tested against each other for cross inoculation. Neither crosses with any of the other in the list.

literature and several preliminary trials, a 2 per cent sodium chloride solution was selected as the extracting agent. The seeds were ground to a fine meal and extracted with the saline solution in the proportion of 1 part of meal to 10 of solution. The extractions were continued for 30 minutes at room temperature, and then filtered

clear through a mat of paper pulp. The clear filtrate was used as the antigen, and fresh extracts were prepared each time the material was used.

PRECIPITIN REACTION

Antisera were prepared for the saline extracts of the seeds of spring vetch, garden pea, alsike clover, white clover, garden bean, lima bean, Japan clover, yellow lupine, serradella, sanfoin, soy bean, kidney vetch, fenugreek, alfalfa, and dalea. Rabbits were used in the preparation of the antisera. The injections were made intraperitoneally. With the exception of the kidney vetch, nine injections were made, comprising a total of 45 cc. of antigen in each case. With kidney vetch ten injections and 50 cc. of antigen were used. In every case the rabbits remained in good condition and steadily gained in weight.

Preliminary tests were carried out to determine the value of removing the albumen in extracts by heat coagulation. Extracts were heated to 60° C. for 30 minutes and the coagulum filtered off. No significant difference between heated and unheated extracts was noted, and the unheated extracts were used in all the tests reported here.

The animals were aseptically bled from the heart and the serum collected and stored under sterile conditions in the ice box. In cases where there was any doubt as to the presence of bacteria in the sera, phenol was added to make a 0.5 per cent concentration.

For the precipitin test, the antigen was diluted in concentrations ranging from 1-100 to 1-51,200. To 1 cc. of the diluted antigen in a 9 mm. test tube, 0.1 cc. of undiluted serum was added. The two solutions were thoroughly mixed and incubated at 37° C. for 12 hours. Care was taken to keep the materials free from contamination and no trouble was experienced from bacterial growth in the tubes during the incubation period. Controls were carried out in every case. Each antiserum was tested against antigens prepared from twenty-nine different legumes, comprising members from at least nine different cross inoculation groups. Tables II and III record the results of these tests. Table II gives as an illustration the complete data which were secured with the spring vetch anti-serum. Table III summarizes the data from all the experiments.

TABLE II
GROUPING OF LEGUMES ACCORDING TO PRECIPITIN REACTION WITH THEIR SEED PROTEINS
SERUM VENUS ANTISERUM

TABLE III
GROUPING OF LEGUMES ACCORDING TO PRECIPITIN REACTION WITH SEED PROTEINS (HIGHEST DILUTION OF ANTIGEN WHICH GAVE PRECIPITATION IS LISTED; MOST OF RESULTS AT DILUTIONS OF 1600 AND ALL BELOW THAT DILUTION ARE OMITTED)

ANTIGENS FROM SEED OF	ALFALFA	FENUGREEK	ALSIKE CLOVER	WHITE CLOVER	SPRING VETCH	GARDEN PEAS	LIMA BEAN	JAPAN CLOVER	GARDEN BEAN	SOY BEAN	YELLOW LUPINE	SERRADELLA	SANFOIN	DALEA	KIDNEY VETCH
Alfalfa.....	12800	6400
White sweet clover.....	12800	6400
Huham clover.....	12800	6400
Fenugreek.....	6400	12800
Red clover.....	6400	6400
White clover.....	6400	6400	6400
Alsike clover.....	6400	6400	6400
Garden pea.....	6400	12800
Canada field pea.....	6400	12800
Broad bean.....	6400	6400
Hairy vetch.....	6400	12800
Spring vetch.....	12800	12800
Wild vetch.....	12800	12800
Cowpea.....	3200	1600
Lima bean.....	12800	3200
Japan clover.....	1600	6400
Velvet bean.....	3200	3200
Beggarweed.....	3200	3200
Scoich broom.....	3200	6400
Purze.....	3200	6400
Garden bean.....	25600	12800
Soy bean.....	1600	6400	6400	3200
Yellow lupine.....	1600	6400	6400	3200
Blue lupine.....	1600	6400	6400	6400	6400	3200
White lupine.....	1600	6400	6400	6400	6400	6400	1600	3200	3200	3200	3200
Serradella.....	1600	1600	1600	1600	1600	1600	1600	3200	3200	3200	3200
Sanfoin.....	1600	1600	1600	1600	1600	1600	1600	12800	12800	12800	12800
Dalea.....	1600	1600	1600	1600	1600	1600	1600	3200	3200	3200	3200
Kidney vetch.....	1600	1600	1600	1600	1600	1600	1600	3200	3200	3200	3200

Spring vetch and garden pea antisera react much alike; in each case garden pea, Canada field pea, broad bean, hairy vetch, spring vetch, and wild vetch are placed in one group which is quite distinct from all other legumes tested. It will be noted that these legumes also belong to a single cross inoculation group. Most of them have been used by previous workers and similar results secured.

The alsike and white clover antisera appear to be almost identical. In each case red, white, and alsike clover are placed together in one group, considerably separated from any of the other legumes tested. This group likewise corresponds to the cross inoculation group, and also checks with the previous work which has been done with these plants.

With sanfoin antiserum the homologous antigen reacted in a dilution of 1-12,800; yellow lupine, blue lupine, and serratella proteins reacted in a dilution of 1-3200; and velvet bean, beggarweed, and white lupine protein reacted in a dilution of 1-1600. As measured by the cross inoculation tests sanfoin belongs by itself, and the precipitin tests might readily be interpreted to substantiate this. According to the precipitin test, the members of the lupine cross inoculation group are closely related; also velvet bean and beggarweed appear somewhat closely related.

The antisera prepared for yellow lupine and serratella react much alike; yellow, blue, and white lupine, and serratella are grouped together by this test as well as by the cross inoculation tests. Beggarweed, Scotch broom, furze, and sanfoin also appear to be somewhat closely related. Japan clover and lima bean antisera both group cowpea, lima bean, Japan clover, velvet bean, beggarweed, Scotch broom, and furze together, and these belong to a single cross inoculation group. The grouping is not nearly so definite as has been seen in certain other cases; in fact, members of the lupine group and dalea appear as closely related to Japan clover as does cowpea. This cross inoculation group represents a wide range of species, from the standpoint of botanical classification, and it may be regarded as remarkable that this close relationship is exhibited in regard to protein characteristics.

With garden bean antiserum the homologous protein reacted in a

dilution of 1-25,600, while the highest reaction dilution of any heterologous protein was 1-1600. This separation checks the results of other workers, and also corresponds to the cross inoculation group. The tests with soy bean antiserum place soy bean in a class by itself. The homologous protein reacted in a dilution of 1-12,800, while the highest reacting dilution of any heterologous antigen was 1-1600. This checks with the results of previous work and also with the cross inoculation group.

With kidney vetch antisera the homologous antigen reacted in a dilution of 1-6400. Scotch broom, furze, and dalea proteins reacted in dilutions of 1-3200. With the possible exception of dalea, which has not yet been tested, kidney vetch root nodule bacteria do not inoculate any of the legumes used in this test. The antisera which were prepared for fenugreek and alfalfa react much alike. Alfalfa, white sweet clover, Hubam clover, and fenugreek are placed in a group somewhat well separated from others. This corresponds to the cross inoculation tests.

Dalea antiserum gave a positive reaction with the homologous antigen in a dilution of 1-12,800. Velvet bean, beggarweed, furze, and kidney vetch proteins each reacted in a dilution of 1-3200. Dalea belongs to a cross inoculation group different from any of the legumes used in this test, with the possible exception of kidney vetch.

ANAPHYLAXIS REACTION

As a check upon the results of the precipitin test, many of the proteins were used in anaphylaxis tests. Two methods were used in these experiments, the ordinary gross anaphylaxis one and the uterus strip method of DALE.

In the gross anaphylaxis experiments, guinea pigs of 250-400 gm. weight were injected intraperitoneally with the previously described saline extracts of the legume seeds. After a suitable period of incubation the animals were given intoxicating doses of the various proteins by intraperitoneal injection.

In the uterus strip method of anaphylaxis the technique of DALE (7, 8) was used. The animals were sensitized as in the gross anaphylactic tests. At the end of the incubation period they were killed by a sharp blow on the head, bled by cutting the throat and the two horns of the uterus immediately removed. These were sus-

pended separately in oxygenated baths containing a modified Locke-Ringer solution² which were held at 37°–38° C. by a surrounding water bath. One end of the uterus strip was fastened to a rigid support at the bottom of the bath and the other was fastened to a thread, which passed over a series of pulleys and in turn was fastened to a lever. The end of the lever held a needle point which was made to impinge upon the surface of a smoked paper upon the revolving drum of a kymograph. After a relatively short time (20–30 minutes) the uterus strip assumed a regular rhythmic contraction and relaxation. When this occurred one of the protein extracts was added to the Locke-Ringer bath; 1 cc. of protein extract in 50 cc. of Locke-Ringer solution. The addition of the homologous protein causes a very decided contraction of the muscle, persisting over a considerable period of time and inhibiting the regular rhythmic contraction and relaxation. The addition of a heterologous protein causes no such reaction in the tissue.

A piece of tissue which has been saturated with the homologous antigen will not give another reaction upon subsequent additions of the homologous antigen. The reaction of the tissue in the anaphylaxis test is different in this respect from the general reaction of such tissue to poisons and excitatory agents. In this way it was possible to test out various protein extracts against the sensitized uterus strip and thus obtain a permanent visible record of the course of the reaction.

GROSS ANAPHYLAXIS TESTS

Table IV records the data of two such experiments. In one case the guinea pigs were sensitized with red clover and in the other with kidney vetch. In the red clover set nothing is shown other than the fact that pea is not closely related to red clover in its protein characteristics. In the kidney vetch set, furze is shown to be closely related to kidney vetch, dalea slightly related, and serradella much more closely. The data in both cases support the results of the precipitin tests.

Table V records the results of the cowpea series. Japan clover and lima bean are shown to be rather closely related to cowpea,

² Modified Locke-Ringer solution was made up as follows: sodium chloride 2.00 gm., potassium chloride 0.42 gm., calcium chloride 0.12 gm., dextrose 1.00 gm., sodium bicarbonate 0.50 gm., water, glass redistilled, 1000 cc.

TABLE IV
GROUPING OF LEGUMES ACCORDING TO ANAPHYLAXIS REACTION WITH THEIR SEED PROTEINS

GUINEA PIG NO.	SENSITIZATION		INTOXICATION (DOSE 2 CC.)		SECOND INTOXICATION, AFTER 24 HOURS (DOSE 2 CC.)		PROTECTION
	Protein	Dose (cc.)	INCUBATION (DAYS)	Protein	Reaction	Protein	
21.....	Red clover	1 of 1-100	21	Pea	None	Red clover	Slight
22.....	Red clover	1 of 1-100	21	Red clover	Moderate	Red clover	None
23.....	Kidney vetch	1	14	Dalea	Slight	Kidney vetch	Mild
24.....	Kidney vetch	1	14	Dalea	Slight	Kidney vetch	Mild
25.....	Kidney vetch	1	14	Furze	Severe, dead	Kidney vetch	None
26.....	Kidney vetch	1	14	Furze	Severe, dead	Kidney vetch	Moderate, severe
27.....	Kidney vetch	1	14	Serradella	Very mild	Kidney vetch	Moderate, severe
28.....	Kidney vetch	1	14	Serradella	Very mild	Kidney vetch	None
29.....	Kidney vetch	1	14	Kidney vetch	Severe	Kidney vetch	Complete
30.....	Kidney vetch	1	14	Kidney vetch	Severe	Kidney vetch	Complete

TABLE V
GROUPING OF LEGUMES ACCORDING TO GROSS ANAPHYLAXIS REACTION WITH SEED PROTEIN⁵

GUINEA PIG NO.	SENSITIZATION WITH COWPEA PROTEIN (DOSE 1 CC. OF 1-10)	INCUBATION PERIOD (DAYS)	INTOXICATION (DOSE 2 CC.)		PROTECTION
			Protein	Reaction	
1.....	29	Cowpea	Severe, dead
2.....	29	Cowpea	None	Complete
3.....	29	Japan clover	Very mild	Partial
4.....	29	Japan clover	Very mild	Partial
5.....	29	Lima bean	Mild	Partial
6.....	29	Lima bean	Very mild	Partial
7.....	29	Garden bean	Severe	None
8.....	29	Garden bean	Severe	None
9.....	30	White sweet clover	Severe	None
10.....	30	White sweet clover	Severe	None
11.....	30	White clover	Moderate	None
12.....	30	White clover	Moderately severe	None
13.....	30	Garden pea	Severe	None
14.....	30	Garden pea	Severe, dead	None
15.....	30	Cowpea	Moderate	Complete

while garden bean, white sweet clover, white clover, and garden pea bear very little relation to cowpea. This again checks the results of the precipitin tests.

UTERUS STRIP ANAPHYLAXIS TESTS

While the uterus strip method of measuring and recording anaphylactic shock represents an ideal method in many respects, care must be used in the interpretations of results when working with such natural mixtures of proteins as occur in the legume seeds. The degree of sensitization determines to a considerable extent the degree of relationship which will be shown. For example, guinea pig no. 27 (table VI) was so heavily sensitized with lima bean protein, 2 cc. injected, that the uterus strip reacted with sanfoin and yellow lupine proteins practically as strongly as it did with lima bean protein. Previous tests had not indicated any close relationship between these legumes. On the other hand, guinea pig no. C99.3 (table VI) also sensitized with lima bean protein, but with only 2 cc. of a 1-10 dilution, was not sensitized heavily enough to give a reaction with either cowpea or Japan clover proteins, although it did react with lima bean protein. Guinea pig no. 55 (table VI and fig. 1 b), which received 0.5 cc. of lima bean protein, reacted with both cowpea and Japan clover proteins as well as lima bean protein, and this checks with both the precipitin and the cross inoculation tests. Tables VI and VII give the results of these tests and figs. 1 and 2 show photographs of a few of the charts. In the main these results check closely with the results of the precipitin and gross anaphylaxis tests. Due to individual variations in the degree of sensitization, however, groups in some cases might be enlarged and in other cases made smaller, if based entirely on the results of this test.

With guinea pig no. 17 the close relationship between broad bean, garden pea, and spring vetch is indicated. In fig. 2 the charts *g* and *h* produced with this pig are reproduced. The pig was sensitized with broad bean antigen and the addition of cowpea and lima bean antigens produced no reaction, while the addition of spring vetch antigen caused a marked contraction of the uterus strip with a temporary inhibition of the rhythmic contractions and relaxations. This addition of spring vetch antigen was enough to saturate the

TABLE VI
GROUPING OF LEGUMES ACCORDING TO THE ANAPHYLAXIS REACTION WITH SEED PROTEINS
(TERTIUS STRIP METHOD); INCUBATED 14 DAYS

GRINNE PG NO.	SENSITIZATION		INTOXICATION				
	Protein	Dose	First	Second	Third	Fourth	Sixth
17.....	Broad bean	2 of 1-10	NaCl —	Garden bean +	Cowpea —	Garden pea +++	Broad bean —
17.....	Broad bean	of 1-10	Cowpea —	Lima bean —	Spring vetch +++	Spring vetch —
29.....	Cowpea	0.5	Garden pea —	Furze +	Cowpea —
29.....	Cowpea	0.5	Sanfion +	Furze +	Lima bean ++
29.....	Lima bean	2.0	Sanfion +	Yellow lupine +	Lima bean —
55.....	Lima bean	0.5	Cowpea +	Cowpea —	Sanfion —
C 99.1.....	Lima bean	2 of 1-10	Japan clover —	Cowpea —	Lima bean ++
C 99.1.....	Lima bean	2 of 1-10	Garden bean +	Cowpea —	Lima bean ++
C 99.1.....	Lima bean	2 of 1-10	Garden pea —	Japon clover —
C 99.1.....	Lima bean	2 of 1-10	Garden bean —	Broad bean —
C 99.1.....	Lima bean	2 of 1-10	Garden bean —	Broad bean —
34.....	Beggarweed	0.5	Garden pea —	Velvet bean —	Lima bean —	Scotch broom —
34.....	Beggarweed	0.5	Garden pea —	Velvet bean —	Cowpea —	Scotch broom —
13.....	Furze	0.5	Blue lupine —	Sanfion —	White sweet clover —	Scotch broom ++
13.....	Furze	0.5	Garden pea —	White clover —	Cowpea —	Scotch broom ±
5.....	Scotch broom	0.5	Japan clover ±	Japan clover —	Serradella —	Dalea —
5.....	Scotch broom	0.5	Beggarweed ++	Beggarweed —	Santolina +	Kidney vetch +	Furze +++
6.....	Scotch broom	1.0	Sanfion —	Velvet bean —	Furze —	Yellow lupine —
12.....	Yellow lupine	2 of 1-10	Sanfion +	Sanfion +	Santolina +	Blue lupine +
21.....	Yellow lupine	2 of 1-10	Kidney vetch +	Yellow lupine —	Blue lupine ++	Seradella —
35.....	Serradella	0.5	Spring vetch —	Soy bean —	Yellow lupine ++	Soy bean +
38.....	Serradella	0.5	Garden pea ±	Kidney vetch ±	Santolina +	Serradella —

Reaction: —, none; ±, doubtful; +, weak; ++, medium; +++, strong.

TABLE VII
GROUPING OF LEGUMES ACCORDING TO ANAPHYLAXIS REACTION WITH SEED PROTEINS
(UTERUS STRIP METHOD); INCUBATED 14 DAYS

GUNSEA NO.	SENSITIZATION		INTOXICATION					
	Protein	Dose	First	Second	Third	Fourth	Fifth	Sixth
45.....	Kidney vetch	1.0	Serradella ±	Beggarsweed —	Furze + +	Furze + +	Kidney vetch —
		1.0	Hairy vetch ±	White sweet clover —	Furze + +	Kidney vetch + +	Kidney vetch —
		0.5	White sweet clover + +	Beggarsweed + +	Dalea —	Kidney vetch + +	Kidney vetch —
45.....	Crotalaria	0.5	White sweet clover + +	Velvet bean + +	Dalea —	Japan clover —	Japan clover —
		0.5	White sweet clover + +	Kidney vetch +	Yellow lupine —	Dalea —	Dalea —
		0.5	White sweet clover + +	Furze +	Beggarsweed —	Dalea —	Dalea —
10.....	Dalea	0.25	Kidney vetch + +	Furze + +	Japan clover —	Japan clover —	Japan clover —
		0.25	White sweet clover ±	Furze + +	Dalea —	Dalea —	Dalea —
		1.0	White clover —	Beggarsweed —	Furze + +	Furze + +	Furze + +
24.....	Dalea	1.0	White sweet clover —	—	—	—	—
		1.0	White clover —	—	—	—	—
		1.0	White clover —	—	—	—	—
33.....	Huban	0.5	Velvet bean —	White sweet clover + +				
		0.5	Scotch broom —	Huban clover —				
		0.5	Yellow lupine +	White sweet clover + +	White sweet clover + +	White sweet clover + +	White sweet clover + +	White sweet clover + +
41.....	Garden pea	0.5	Garden pea +	Huban clover +	Huban clover +	Huban clover +	Huban clover +	Huban clover +
		0.5	Beggarsweed —	Beggarsweed —	Alfalfa —	Alfalfa —	Alfalfa —	Alfalfa —
		0.5	—	—	—	—	—	—
52.....	White sweet clover	0.5	Scotch broom —	White clover —				
		0.5	Dalea —	—	—	—	—	—
		0.5	—	—	—	—	—	—

Reaction: —, none; ±, doubtful; +, weak; ++, medium; + + +, strong.

tissue, since subsequent additions of both spring vetch and broad bean antigens failed to produce any further reaction. With the other horn of the uterus from this pig, the addition of 1 cc. of a 2 per cent

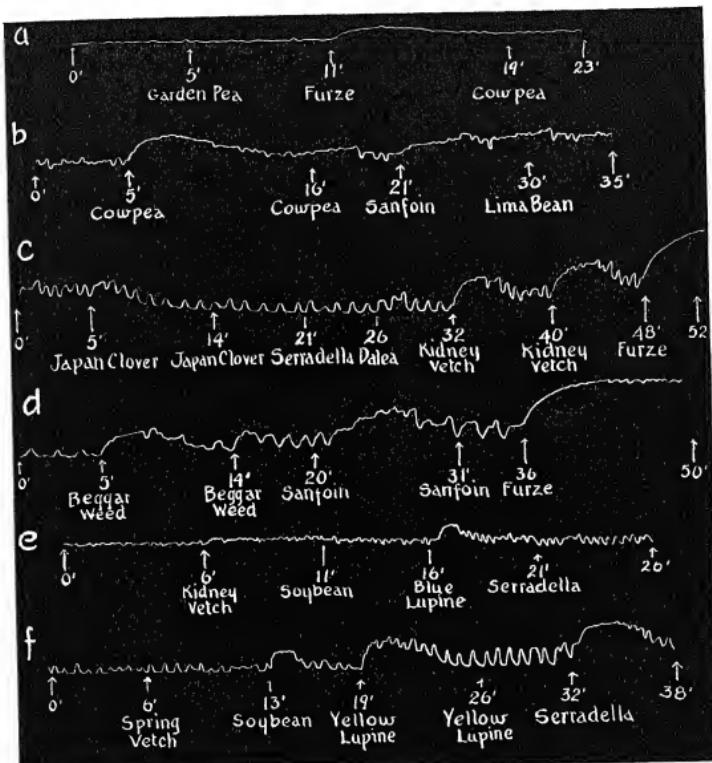


FIG. 1.—Reaction to various protein solutions of uterus strips from guinea pigs sensitized with seed protein from: a, cowpea (guinea pig no. 29); b, lima bean (no. 55); c, d, Scotch broom (no. 5); e, serradella (no. 35); f, serradella (no. 38).

salt solution and of cowpea antigen failed to produce any reaction. The addition of garden bean antigen produced a very slight interruption of the rhythmic contractions and relaxations, while the addition of garden pea antigen produced a pronounced contraction of the uterus strip.

In the reactions of cowpea and lima bean pigs (fig. 1 *a*, *b*) evidence was found which justifies placing cowpea, lima bean, and furze in one group. Furze and Scotch broom (fig. 1 *c*, *d*) are shown to

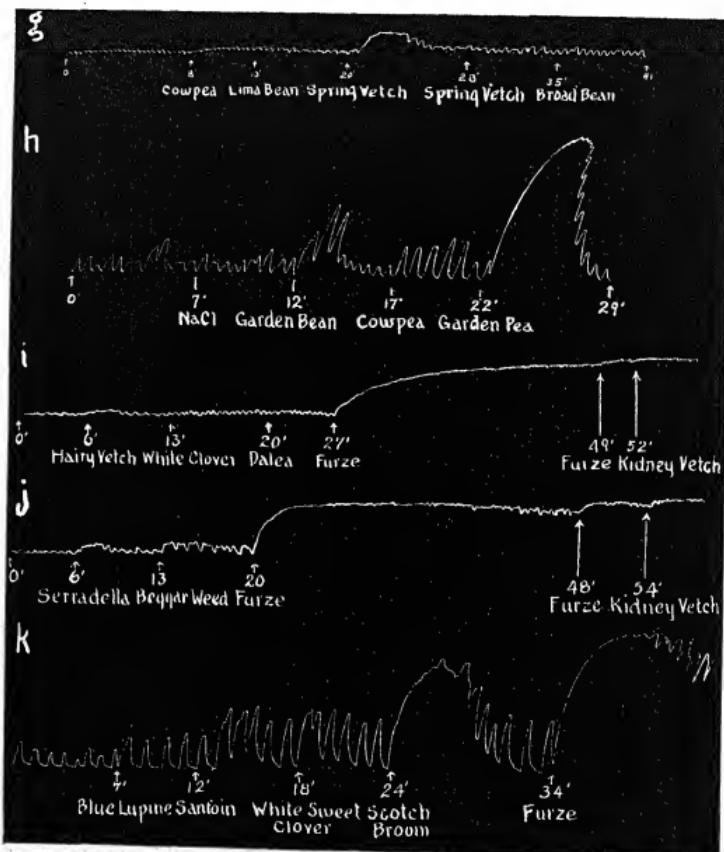


FIG. 2.—Reaction to various protein solutions of uterus strips from guinea pigs sensitized with seed protein from: *g*, *h*, broad bean (guinea pig no. 17); *i*, *j*, kidney vetch (no. 45); *k*, furze (no. 13).

be closely related to each other and to be more distantly related to Japan clover, dalea, kidney vetch, beggarweed, and sainfoin. The chart for guinea pig no. 13 (fig. 2 *k*) gives an example of a case in which a pronounced reaction was secured by the addition of

a heterologous antigen, without giving a saturation effect for the homologous antigen. The pig was sensitized with furze antigen, and the addition of blue lupine, sanfoin, and white sweet clover antigens to the tissue produced no reaction. The addition of Scotch broom antigen gave a decided contraction, but without complete saturation of the tissue for the homologous antigen, since a subsequent addition of furze antigen produced very strong contraction of the tissue. This reaction indicates that while Scotch broom and furze proteins are closely related they are not identical.

The reactions of the yellow lupine and serratella pigs gave ample evidence for the grouping together of the lupines and serratella; however it is also indicated that sanfoin is closely related. Guinea pig no. 45 (fig. 2 *i, j*) indicates the close relationship between kidney vetch and furze, which corresponds with other data which have been secured. The reactions of guinea pig no. C116.3 (table VII) would indicate that hairy vetch, beggarweed, white sweet clover, and velvet bean are each closely related to kidney vetch, and this is not in accordance with other data. The reactions secured with the dalea pigs (table VII) indicate a close relationship between kidney vetch, furze, and dalea, which is in accordance with the data of the precipitin tests. Rather erratic results were secured with the pigs sensitized to white sweet clover (table VII). Frequently extracts of distantly related legumes, as judged by the botanical and precipitin classifications, reacted much more vigorously than did sweet clover itself. With such a wide variety of results, it would hardly be safe to draw any conclusions relative to the legumes most closely associated with white sweet clover.

In general, the results of this test are in harmony with the results of the precipitin and gross anaphylaxis tests.

Agglutination of nodule bacteria with legume protein antisera

SIMON (32) suggested that there might be a relation between the proteins of the legumes and the nodule bacteria, which could be determined by serological methods. No data are reported to substantiate the idea, and there has been no further reference to the subject found in the literature. If the theory of SIMON is correct, one might expect the legume seed antisera to agglutinate the nodule

bacteria from the same species. This was tested and the results indicated that the protein of the nodule bacteria and the legume seed are not closely allied.

Summary

1. A consideration of the factors which might be responsible for the adaptation of certain strains of the legume nodule bacteria to certain species of legumes and not to others suggests that the chemical complex of the host plants may be responsible. It seems reasonable to believe that any two plants, each of which is inoculated with the same strain of root nodule bacteria, must be more or less closely related physiologically. The physiology of any plant comprises a great number of different factors, any one of which might be the controlling one in the matter of inoculation by a specific organism.
2. Similar relationships between the physiological complex of plants and their resistance or non-resistance to invasion and the production of disease have been noted in the case of plant parasites.
3. Additional evidence of the fact that the members of a cross inoculation group in the legumes may be physiologically related is offered by the fact that the cowpea root nodule organism and *Bact. vignae*, the cowpea leaf spot organism, infect exactly the same group of plants.
4. The protein characteristics of plants offer one of the most easily measured factors in their chemistry. Serological methods of measuring the relationships existing between the protein characteristics of plants have been developed and used to a considerable extent. All such studies, which have previously been made of the protein characteristics of legumes, placed them in similar groups to the cross inoculation ones.
5. In the work reported in this paper, the protein characteristics of twenty-nine species of the commonly cultivated legumes have been studied by means of the precipitin and anaphylaxis reactions.
6. The results of these tests show that all members of any cross inoculation group are closely related with respect to the protein characteristics of their seeds, and in the majority of cases all legumes which possess closely related seed protein complexes cross inoculate. The latter point, however, is not true in all cases.

7. Several possibilities suggest themselves to account for these facts. (1) The protein characteristics of the host plant may be the controlling factors in determining whether a particular bacterial type is capable of causing inoculation, and a more careful and detailed analysis of the purified proteins from the seeds of the plant might demonstrate that relationship. (2) If the protein characteristics of the plant are the controlling factors, the protein characteristics of the root, and more definitely of the root hairs, must be the important point, since this is the point of attack by the bacteria. It is probable also that a somewhat different series of plant relationships might be established if the protein characters of the root hairs were taken as the subject for study. (3) It is entirely possible that the protein characters are not the controlling factors in the matter of inoculation by the root nodule bacteria, and that the protein relationships shown to exist between members of the cross inoculation groups are correlated to a certain extent with another physiological factor which is the controlling one.

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BEHAVIOR OF CERTAIN FERN PROTHALLIA UNDER PROLONGED CULTIVATION

DAVID M. MOTTIER

(WITH PLATE X AND SEVEN FIGURES)

At the Cincinnati meeting of the Botanical Society of America, in December 1923, the writer gave a preliminary report upon the behavior of the prothallia of *Matteuccia nodulosa* (Michx.) Fernald (*Onoclea Struthiopteris*), and *Osmunda claytoniana* L. under prolonged cultivation. The data presented at that time were based upon prothallia that were a little more than a year old in the case of *M. nodulosa*, and seven months old in that of *O. claytoniana*. A number of the same prothallia have been kept growing, so that the results of four years of continuous observations are now at hand, and the more important features of these will be set forth in the following paper.

Historical

The ordinary behavior of these prothallia in culture is so well known that a description of their development from the spore will not be repeated. In the literature one finds references to old prothallia of the Polypodiaceae and Osmundaceae, but the writer has not been able to find any record of continuous growth in culture from the time of sowing to the end of the fourth year. It is well known that the prothallia of the two genera under consideration, as well as those of other Polypodiaceae, when grown beyond the ordinary time for the production of sporophytes, may branch both dichotomously and by means of lateral proliferations. The proliferations may spring from both the upper and the under sides of the midrib (MOTTIER 9), and from the margins of the wings of the gametophyte.

HOFMEISTER (6) calls attention to lateral proliferations or adventitious shoots in the prothallia of *Notochlaena*, *Allosurus*, and *Gymnogramma calomelanos*. Similar structures have been described later by KNY (7), GOEBEL (3), CAMPBELL (1, 2); and doubtless by many other observers. KNY, in referring to *Osmunda regalis*, states that the prothallia produce adventitious shoots in a high degree.

These arise from the margin of the wings and not from the surface. In a footnote, however, he mentions a single case in which a shoot sprang from the midrib on the under side, and upon this shoot numerous archegonia were developed. He regarded the shoot as an abnormality. GOEBEL (3) has called attention to the production of numerous and varied adventitious shoots from both young and older or mature tissues of the prothallia of *Gymnogramma leptophylla* Desv. In older prothallia shoots arise either from the edge or from the surface. In regard to the dichotomous branching of prothallia, the earliest account that the writer has found is that of GOEBEL (3), who described and figured this mode of branching in *Osmunda regalis*. The dichotomy, which occurs rarely, takes place by the regular bifurcation of the growing point. In speaking of the branching in *Osmunda*, CAMPBELL (1) states:

Not infrequently, especially in *O. claytoniana*, the young prothallia branch irregularly, or in some cases there seems to be a true dichotomy (*I.c.* fig. 21); but in the latter case one of the branches finally grows faster than the other, which is suppressed, and the resultant prothallium does not differ much in appearance from the ordinary type.

CAMPBELL calls attention also to "secondary prothallia" in *Osmunda*, which may arise from the margin and from the lower surface. By the death of the older tissue these shoots become isolated, continue to grow, and produce normal sexual organs.

In regard to the continued growth of old prothallia that do not bear sporophytes, GOEBEL (3) refers to a much branched four-year old prothallium of *Osmunda regalis* that had attained a length of 3-4 cm., resembling in appearance the liverwort *Pellia epiphylla*. He states further that among these prothallia old Polypodiaceous prothallia were found exhibiting the same habit of growth. He did not mention the species referred to, and no data as to the method of cultivation were given. As will be shown in a following paragraph, prothallia of *O. claytoniana* may attain a length of 3.5 cm. in fourteen months.

Method of culture

Stock cultures of the prothallia were first made upon sterilized woods earth, and as soon as the plants had attained a width of 2-4 mm. across the wings, the most vigorous individuals were trans-

planted to separate vessels. In all cases, unglazed earthenware flower pot saucers 5 or 6 inches in diameter were used. Each culture was covered with a bell glass, which was usually propped up on one side to a height of about one-fourth of an inch to insure ventilation and to facilitate evaporation, should the earth be made too wet in watering. Cultures were always watered by sub-irrigation. A more detailed account of the method of culture has been published elsewhere (8). From twenty to twenty-five prothallia can be conveniently spaced in each saucer. Text figs. 1, 2, and 6 show the appearance of the transplanted prothallia from four to seven months old.

In order to grow prothallia for a long period of time, it is necessary of course to prevent the production of sporophytes, and to amputate, without injury to the prothallia, such sporophytes as may develop in spite of precautions. Since the plants were watered by sub-irrigation, sporophytes rarely appeared, especially in older plants. After a little practice a sporophyte possessing the first visible leaf may easily be amputated close to the surface of the prothallium with a sharp, lance-shaped needle or the blade of a safety razor. When the operation is successfully done, scarcely any traumatic effects result, and such plants continue to grow as do those upon which no sporophytes have developed. For reasons that will be mentioned later, sporophyte production is more easily prevented in old prothallia.

Prothallia of other species are more easily grown than those of the two selected, but under the greenhouse conditions available it was necessary to select species whose prothallia could always be distinguished from "weed" prothallia that almost invariably find their way into the cultures. Even in cultures grown on outside window shelves of the laboratory, far removed from other ferns, "weed" prothallia made their appearance, having developed from wind-blown spores. The writer has always been able, with the aid of a simple magnifier, to distinguish between the prothallia of *Matteuccia nodulosa* and *Osmunda claytoniana* and those of other ferns grown in the greenhouse, as well as those from other sources. Diffused light in both winter and summer proved most favorable for the growth of the plants, although direct sunlight for a short period in early morning did not seem to be unfavorable.

Growing fern prothallia for a short period is a comparatively easy task, since few foreign plants will tend to appear on sterilized soil, especially if the prothallia are crowded; but when cultivation is extended through months and years, many difficulties confront the experimenter. Chief among the trouble makers are moss protonema, which tend to form a mat on the surface of the soil, unicellular green algae, and certain soil-loving blue-green algae, as species of *Lyngbya*, and finally fungi and insect pests. Spores and dried parts of the plants mentioned are always in the air, and it is not possible to prevent their gaining access to the surface of the cultures.

Although mosses gain a foothold in the cultures after a time, they are easily held in check. The leafy stems and protonemal mats may be removed from time to time with needle and forceps. When such removal takes along a layer of soil it is necessary to add carefully new sterilized earth. Whenever moss and algal growth became too well established to be held in check by the foregoing method, the prothallia were carefully transplanted to a new dish of sterilized soil. A few times during the past four years it became easier to move the crop, so to speak, from the old to a new field than to try to clean out the weed plants. In some cultures frequent scratching or cultivation of the surface of the soil between the plants was very helpful.

Of the blue-green algae, species of *Lyngbya* were the most troublesome, both in summer and winter. In a few cultures, especially if the soil were permitted to become a little too moist, they tended to form a crust upon the soil, and to spread upon the surface of the prothallia as well. As a remedy the culture was permitted to become somewhat dry, after which the crust was removed and new earth added. *Protococcus* and other unicellular green algae gave little trouble further than to coat over the sides of the earthen saucers.

Of the animal pests, the larvae of a small, gnatlike Dipterous insect were the most troublesome. The small, maggot-like larvae burrow in the soil, feeding upon the young rhizoids and tender parts of the under surface of the thallus. Thus concealed they may do considerable damage before their presence is discovered. At times they come to the surface to devour the tender parts of the thallus above the ground. In crawling over the soil they leave a glistening trail not unlike that of a tiny slug, so that the larvae partly concealed in the

surface of the soil may easily be found with the aid of a magnifier and removed with forceps. In a few cases it became necessary to transplant the prothallia to newly prepared culture dishes. After a thorough renovation of the greenhouse in midsummer this pest disappeared. Attacks of fungi, which occurred occasionally in a few cultures, gave little trouble. As soon as a moldlike growth appeared, the infected part was removed and destroyed. During the entire four years but one small culture was lost because of fungal growth.

Observations

MATTEUCCIA NODULOSA.—Although the behavior of the prothallia of the two species was similar in many respects, that of each will be given separately. As stated in the foregoing, as soon as the more vigorous specimens in the stock culture were large enough to handle easily (2-4 mm. diameter), they were carefully transplanted to separate cultures, being spaced 2 or 3 cm. apart. If the plants touched one another or became crowded because of increase in size, a number of individuals were again transplanted to other saucers. Text fig. 1 illustrates the method of culture, and shows the prothallia four months old when grown under these conditions. In cultivation the object desired was to grow the plants upon earth under the most favorable conditions that could be provided. Only the more vigorous and rapidly growing specimens were selected in every instance. Any one who has examined fern spores carefully, and who has grown prothallia extensively, is impressed with the marked variation in the size of the spores; the readiness with which some germinate, and the rapidity and vigor of growth of certain prothallia. Presumably the larger and more rapidly germinating spores are those that produce the more vigorous prothallia.

At the end of about four months from the date of sowing (October 11, 1922 to February 28, 1923), the largest prothallia had attained a width of 15 mm. (text fig. 1). These plants had a relatively broad, massive midrib (archegonial cushion), and broad, rounded wings. At this age the wings are noticeably ruffled or wavy. In a number of plants there developed from the anterior margin of the wings, near the apical sinus, small thin, colorless, scalelike outgrowths, which generally curved upward and backward, or became variously twisted.

They were chiefly one cell in thickness and contained little or no chlorophyll. In the photograph they appear white and are easily recognized. After a time such scalelike outgrowths wither and disappear. They appeared now and then in older prothallia, but with no marked regularity. In character they suggest the minute pinkish scales borne along the margins of the thallus of *Marchantia*, although



FIG. 1.—*Matucaria nodulosa*: culture of prothallia four months old, grown upon woods earth; natural size.

less uniform in size, and appearing much less regularly and in smaller numbers.

Text fig. 2 shows eight prothallia of the same culture when seven months old. In this culture, the only one in which the phenomenon occurred, a few plants developed a spinelike outgrowth from near the apical sinus (the central plant in the row of three at the right). These outgrowths were about 5 mm. in length, cylindrical or somewhat flattened, and tapering somewhat gradually to a sharp point. In structure they consisted of narrow, elongated parenchyma cells poor in cytoplasm. Near the center of each of those sectioned with the

microtome was a short strand of slender spiral tracheids three cells in width and four cells in length. The strand of tracheids was located at about one-third the distance above the base of the spine. No tracheids were present in the base of the spine, although that was its thickest part, nor in the midrib from which the spine developed. In a short time the spinelike outgrowths withered and disappeared, and no others were developed on the same plants afterward.

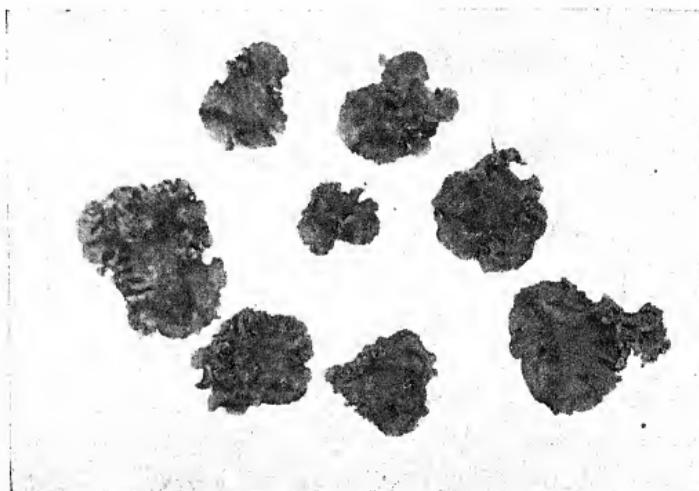


FIG. 2.—*Matteuccia nodulosa*: Prothallia seven months old; central plant in row at right shows spine-shaped outgrowth; natural size.

Of the plants grown, but three developed similar outgrowths. They were the only suggestions of anything that might be regarded as a tendency toward an apogamous structure. In the absence of a knowledge of their complete history and histological structure, one might easily be led to conclude that apogamy occurred here. That the structures withered in a comparatively short time, and occurred in but few instances, does not justify, from this evidence alone, the conclusion that apogamy occurs in *Matteuccia nodulosa*. These cases have convinced the writer that the presence of tracheary tissue alone in the prothallia is not proof of the existence of apogamy. If the prothallia of *M. nodulosa* had any tendency to develop apogamous

sporophytes, favorable conditions were certainly present for them to do so. The larger prothallia of text fig. 2 show the beginning of the characteristically ruffled margins of the wings, and the appearance of the heart-shaped proliferations that spring from the surface and margins of both young and old parts of the plants.

When the prothallia of *M. nodulosa* have attained the sizes of those of text figs. 1 and 2, from seven to eight months from the time of sowing the spores, their subsequent development results in one or the other of two forms of growth described by earlier observers. They may branch dichotomously, as in figs. 1, 2, and 6, or they may give rise to very irregular expansions, as seen in fig. 7. In some cases they produced dichotomous branches sparingly, with the result shown in fig. 4, although in this and in similar cases proliferations are beginning to develop at the proximal ends. Prothallia with the growth-habit of fig. 1, are of theoretical interest because of their resemblance to certain dichotomously branched thallose liverworts. At a glance the plants might easily be taken for such liverworts as *Pellia*, a fact frequently mentioned by the earlier observers. At the end of four months' growth this plant had branched dichotomously, and subsequently each of the two shoots branched again in the same manner. A massive midrib, or archegonial cushion, leads to each growing end, which is relatively broad. The margins or wings of each shoot are quite regularly ruffled, and the branches tend to overlap to some extent. On the under side of the midrib or midribs, numerous archegonia are regularly and continuously developed. At the margins of such prothallia as shown in figs. 1-6, numerous proliferations are formed, which bear antheridia in great numbers. These proliferations are too small to show distinctly in the photographs. In addition to the marginal antheridia-bearing proliferations, small granular ones appear on the dorsal surface of the midrib, especially of the older parts. At the older proximal ends of figs. 6 and 7 may be seen rounded, wartlike outgrowths of a granular appearance, covered with antheridia. In some cases, when the older parts of the midrib had begun to turn brown as if dead, small antheridial proliferations developed from the dark brown surface. While the older parts of the midrib appear dead at the surface, the cells below the epidermis remain meristematic for a longer time. Death of the older proximal

parts of the prothallia is slow and gradual when growing conditions are the best, and may not begin until the close of the second year. The behavior of the plant shown in fig. 6 differed somewhat from that of fig. 1. At the age of ten months it consisted of six definite growing points, resulting from dichotomous branching.

The second growth habit is to be seen in figs. 3, 5, and 7, where the branching is much less regular than dichotomy, the result being a very irregular form. Fig. 3 represents the plant at the age of ten months, while fig. 7 is the same plant at the age of one year. In so far as the writer could ascertain, these plants were not injured in any manner. A comparison of figs. 7 and 7a will indicate strikingly the size that may be attained in one year's growth under favorable cultural conditions when sporophytes are not developed. The plant represented in fig. 7 is the result of both dichotomy and larger lateral proliferations. Such lateral proliferations spring either from the margins of the wings or from the midrib. In the literature they have been referred to as adventitious shoots. In this, and in other similar prothallia, numerous small, heart-shaped proliferations are developed, in addition to the larger and more vigorous shoots. Most proliferations that attain any considerable size begin as heart-shaped outgrowths. Fig. 7a is a prothallium about six weeks old, bearing a young sporophyte consisting of the first visible leaf and root. Under ordinary conditions prothallia usually produce sporophytes when 2-4 mm. in diameter. The area of the prothallium in fig. 7a is close to 9 sq. mm., while that of fig. 7 is 1700-2000 sq. mm., not counting the smaller proliferations.

The plant shown in fig. 8 was photographed a little less than natural size when two years and three months old. Up to this time it had not grown as rapidly as some others, but from this time on growth was vigorous. The photograph was taken January 24, 1925. During the previous December the plant grew slowly because of unfavorable greenhouse conditions. In its earlier growth it developed three shoots which grew in as many different directions. The oldest parts have died, the three shoots being still connected by the dead strips of old midribs. In all prothallia the older parts die and decay sooner or later, as in liverworts. In some cases the death of the old-

est parts did not begin until about the end of the second year, but there is much variation in this feature, as would be expected.

In the plant in question (fig. 8) the lower left part consists of five vigorous shoots or branches, each with a relatively broad, massive midrib, and much ruffled margins. The part second in size (the upper in the figure) has three branches, and the smallest part (at the right) has two distinct growing points. In these shoots the branching seems to have been of the dichotomous type, although lateral shoots arise behind the growing edge or apical sinus. The growth habit here is almost a perfect imitation of that of a thallose liverwort. In this, as in almost all other prothallia of this species, many lateral proliferations arise from the margins of the ruffled wings. The smaller of these bear numerous antheridia. Archegonia are confined largely to the under surface of the midrib, while few or no antheridia may develop there. For this reason fecundation is very easily prevented.

Three months later (April 24) the three parts, continuation shoots, were completely isolated, the proximal portions of the midribs having completely decayed. The smaller part grew less rapidly than the other two. The surface now covered by the three shoots, with the exception of the central area resulting from the death of the central portion, measured 5.5 by 7.5 cm.; that is, from the spore a prothallial growth equal to those dimensions had developed in two and one-half years. This culture was followed carefully in every detail, in so far as that was possible without injury to the resultant growth. From April to November 1925 these continuation shoots grew rapidly in the manner described, so that by the end of the third year, November 11, 1925, the resultant growth formed a fairy ring or ellipse whose minor axis measured almost 7 cm. and the major axis about 9 cm. (text fig. 3). As will be seen, the shoots are extensively branched, the branches tending to overlap and crowd one another. The growth as a whole continued to spread and enlarge radially in a centrifugal direction, and also centripetally toward the central space left by the death of the older parts. On October 11, 1926, four years from the date of sowing the spores, the continued growth of this one prothallium had spread over an area somewhat circular in outline, 12 cm. in diameter (text fig. 4). The method of branching is

chiefly dichotomous, the older proximal portions gradually dying. I have spoken of the branching as the dichotomous type, for it is the growing point, or apical sinus, that bifurcates in a manner described by earlier observers. In addition, branches of the prothallia of the ferns in question arise frequently also by proliferations from the margins and from the upper and lower sides of the midrib.

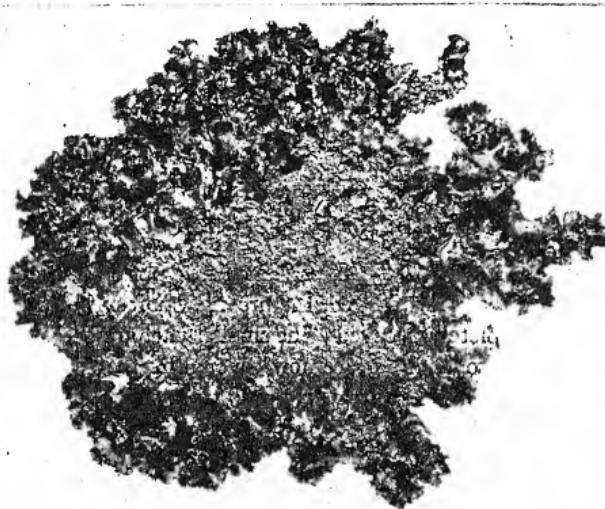


FIG. 3.—*Matteuccia nodulosa*: fairy ring of continuation shoots developed from single prothallium at end of third year; natural size.

As may be seen from the photographs with a certain degree of clearness, the more vigorous shoots are about 10 mm. wide, with midribs 3-4 mm. wide. When the shoots are not crowded they become broader, as shown in text fig. 4. The entire growth is now represented by the three large separate masses, all the shoots of each still histologically connected. In this culture the continuation growth consists of numerous shoots with definite midribs. In places the shoots crowd and overlap to a large extent. This leads to a more rapid death of older parts. Text fig. 5 shows the habit of growth which is typical of the continuation shoots of *M. nodulosa*, at the end of the fourth year. It represents the result of one of the shoots of a parent

prothallium which became independent through the death and decay of older proximal parts.

One prothallium of *M. nodulosa*, therefore, if kept growing for four years on soil and not allowed to develop sporophytes, will produce a plant body approximately equal to a surface 120 mm. square, 14,400 sq. mm., or 1600 times as large as a prothallium which bears a

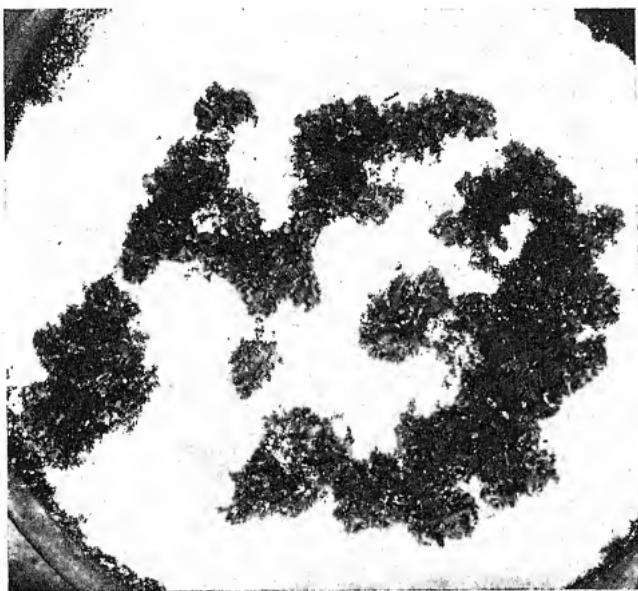


FIG. 4.—*Matteuccia nodulosa*: surviving continuation shoots resulting from single prothallium at end of fourth year; $\times \frac{3}{4}$.

sporophyte under ordinary conditions. The actual mass of the plant body produced is probably much greater, owing to the overlapping of the many lobed and ruffled shoots. Each shoot seems to be capable of continuing growth in somewhat liverwort fashion for an indefinite time.

OSMUNDA CLAYTONIANA.—The general behavior of the prothallia in this species was found to be similar to that in *Matteuccia nodulosa*. As pointed out by CAMPBELL (1), they are more elongated than

those of the Polypodiaceae, bearing a resemblance to the liverwort *Dendroceros*. The midrib projects strongly from the lower surface. The irregular branching and the presence of dichotomy, as stated by this author and by GOEBEL, have been referred to in preceding paragraphs.

Text fig. 6 illustrates the habit of growth and the size attained at the end of the first seven months. Even in prothallia of this age the resemblance to such liverworts as *Pellia* and *Dendroceros* is striking. As a rule they do not become as broad and as massive as the prothallia of *Matteuccia nodulosa*. The difference may be seen by comparing text figs. 1 and 6. In fig. 9 are shown four of the plants from



FIG. 5.—*Matteuccia nodulosa*: part of continuation shoots resulting from single prothallium at end of fourth year; natural size.

the culture represented in text fig. 6, at the age of fourteen months. GOEBEL (3) speaks of a four-year old prothallium of *Osmunda regalis* that had attained a length of 4 cm. The largest plant in fig. 9 measured nearly 3.5 cm. in length, and the very unusual prothallium shown in fig. 11 had a length of 5 cm. at the age of twenty months. Here, as in *M. nodulosa*, one recognizes two types of development: that of a broad, irregular expansion, which is comparatively rare, however, and the less branched habit in which the larger shoots or proliferations are fewer. The former type of development is shown in figs. 10 and 11, and the latter in fig. 9. Dichotomous branching occurs in old prothallia, but it is less frequent than in *M. nodulosa*. The two shoots resulting from the dichotomy grow unequally as a

rule, so that one becomes much larger than the other. As a result, therefore, we do not have the regular symmetrical habit seen in figs. 1 and 2, but that which is more like fig. 10.

The most remarkable plant in the culture of *O. claytoniana*, as regards habit and vigor of growth, is represented in fig. 11. This figure is a photograph, natural size, of the plant when twenty months old. It consisted of numerous large and small branches, more or less crowded and overlapping. All the midribs were alive and connected histologically, so that the whole consisted of one much branched

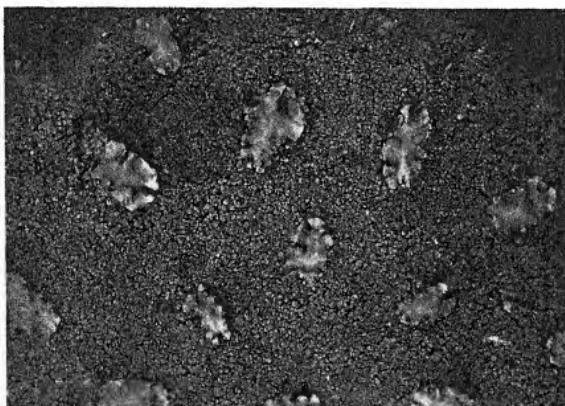


FIG. 6.—*Osmunda claytoniana*: several prothallia from culture seven months old; natural size.

prothallium. Each larger branch has developed many smaller, heart-shaped proliferations, which give the entire plant the appearance of a thousand or more ordinary prothallia massed together. The larger shoots bear numerous archegonia on the under side, and in some cases on the upper surface as well. Many of the smaller proliferations are thickly beset with antheridia. In older prothallia few or no antheridia are developed on the under side, near the archegonia. Because of this fact fecundation took place very rarely, and its prevention altogether was easily accomplished. When sporophytes appeared occasionally in later stages of this and other plants, they were usually found on the smaller and younger proliferations.

The nature of the larger shoots of this plant is shown by those that extend some distance beyond the margins of the mass. The extreme ruffling of the wings of the shoots is plainly seen in these extensions, as well as in the central part of the plant. The photograph (fig. 11) was taken January 24, 1925. Some weeks after that date the central part of the plant began to turn brown and die. Death at the center continued gradually, and after a few months the plant consisted of a somewhat circular expansion with a dead, brown center. Following the death of the older central part, the shoots continued to grow in different directions, spreading toward the periphery and in the direction of the center. At one time a moldlike fungal growth appeared on a part of the dead central mass, and the affected portion of this central tissue was carefully removed. The mold did not reappear at any subsequent time in this culture.

With continued growth and branching of the main shoots, along with the death of their oldest proximal parts, the entire surviving continuations of this plant at the end of three years and five months, as they appeared in the culture, are shown in text fig. 7. There are three irregular continuation masses or expansions, that have become completely isolated through death of the older parts by which they were once connected in one piece. The extremities of the largest of these irregular continuations will doubtless become disconnected by death of older parts at a later date. At the present writing the many shoots of these expansions show a fresh, vigorous growth. If the larger of these shoots were isolated carefully, they would be seen to resemble closely in habit the younger prothallia shown in fig. 9. The under side of each midrib continues uninterruptedly to develop numerous archegonia. Antheridia, although produced sparingly or not at all on the under side, are present in large numbers at the edges and on the surface of the small lateral proliferations of the wings. These antheridia-bearing proliferations, often very irregular in form, are frequently as small as, or smaller than the grains of white sand used in covering the bare soil of the culture before the photograph was taken. To the unaided eye such proliferations are often of a paler or yellowish green color, and when numerous and closely aggregated they appear somewhat as granular masses.

The continuation shoots of the four-year old cultures derived

from a single prothallium show about the same habit as that in fig. 9. Many are a little larger than those in this figure, with a midrib 2-3 mm. in width, and a total breadth of 7-8 mm. They contrast strikingly with the largest continuation shoots of *M. nodulosa*, in which the total width may exceed 12 mm., the midrib measuring 3-5 mm. An especially noticeable and characteristic feature of the continua-

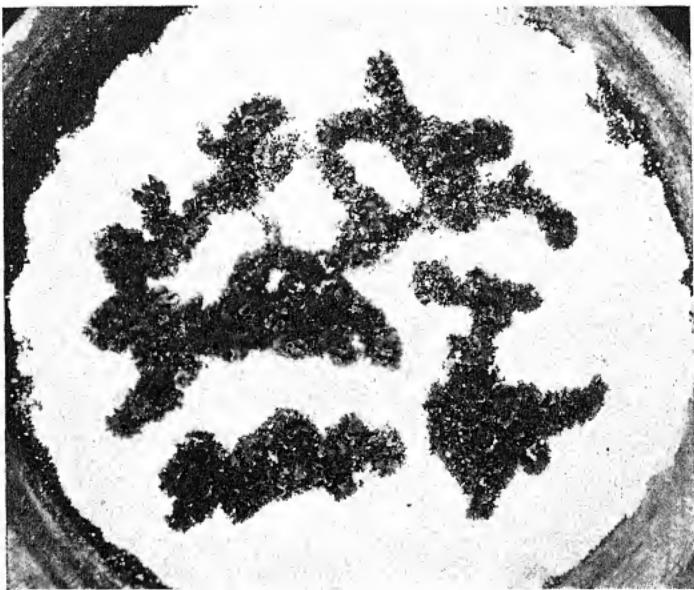


FIG. 7.—*Osmunda claytoniana*: continuation growth developed from prothallium at end of three years and five months; $\times \frac{1}{2}$.

tion shoots of *O. claytoniana* is the pronounced ruffling of the wings, which in some cases may stand up at right angles to the midrib. The edges of some of the lobes, therefore, point vertically upward. In such cases the lobes on opposite sides conceal the midrib. Under such circumstances the upper surface of the midrib will frequently develop a row of minute wartlike protuberances which bear archegonia. These are developed also on the under side of such midribs; antheridia, however, appear on the uneven edges of the ruffled

wings. The edge of each lobe of the ruffle is uneven, and it is mainly from the margin of the uneven edges that antheridia develop. Archegonia frequently appear on the upper side of the midrib in certain prothallia when such part is not shaded by the upstanding or over-arching wings. In a few cases, in which crowding caused certain shoots to grow upright, with the growing point extending straight up into the air, both archegonia and numerous brown rhizoids appeared on both surfaces of the midrib.

In the two species under discussion there seems to be no reason to believe that the continuation shoots resulting from one prothallium at the end of the fourth year could not continue to grow for an indefinite time if kept free from insect pests, from the more vigorous competitors that gain a foothold in the cultures, and from parasitic fungi. Although the plants of some of the cultures have died, as was to be expected, many have been successful, and the continuation shoots of these give at the present no indication of impaired vigor.

Endophytic fungus

An endophytic fungus has been found to be present in the following species of the ferns with green prothallia, as reported by CAMPBELL (2). *Marattia Douglasii* Baker, *Kaulfussia aesculifolia* Bl., *Angiopteris evecta* Hoffm., *Gleichenia polypodioides* Sm., *G. dichotoma* Willd., *G. laevigata* Hooker, *G. pectinata* Presl., and *Osmunda cinnamomea* L. To this list the writer is now able to add *Osmunda claytoniana* L.

CAMPBELL states that in *O. cinnamomea* the endophyte appears commonly, but it could not always be found. In three-year old prothallia of *O. claytoniana* the writer found an endophytic fungus in the older tissues. Of the three specimens imbedded and sectioned, the fungus was present in two but absent from the third. Until the cultures were four years old I did not wish to sacrifice the plants for histological study; however, a somewhat more careful study of their structure will be made in the future. In the four-year old prothallia of *Matteuccia nodulosa* no endophyte has been found in any of the plants examined. It seems probable that the endophyte may be present in this species also. In prothallia of *O. claytoniana* the fungus seems to be confined to circumscribed areas in the midrib. It does not occupy a uniform layer nearer the ventral surface, but its distri-

bution in the specimens observed suggests that of the colonies of *Nostoc* in the gametophyte of *Anthoceros*. The region containing the fungus may be midway between the upper and lower surfaces of the prothallium, or nearer the under surface.

The hyphae are relatively large, tubular, branched, and multi-nuclear. The structure and appearance of the fungus agree closely with that figured for *O. cinnamomea* by CAMPBELL (2). Conidia were not found. In cells containing the fungus the plastids are strikingly different from those in the neighboring cells free from the endophyte. In the former the plastids tend to aggregate into irregular, lumpy masses. They do not contain any starch inclusions, but possess dense and very finely granular contents, which stain deeply and uniformly throughout. With the triple stain of anilin safranin, gentian violet, and orange G (dissolved in clove oil), these plastids retain the safranin strongly, but the violet is easily washed out. As a rule the infected cells are very poor in cytoplasm. The nuclei in some cases seem normal, but in many cells near the center of the infected region the nuclei are ameboid in shape, and appear abnormal in structure. The contents of the fungal filaments differentiate readily in staining, the small nuclei being well defined, and the granular cytoplasm retaining the blue color a little more strongly than the red.

The cell walls of the older parts of the midrib of the prothallium are much thicker than those near the growing point, and are perforated by numerous pits varying much in size. The pits are round, oval, or elliptical; some are elongated and slitlike. They are not arranged in any definite order in the wall. The fungal filaments pass from cell to cell through the larger pits. The fungus was found in some of the rhizoids, so that it seems probable that it gains entrance partly through the rhizoids. Whether we have here a case of parasitism or one of symbiosis is a question. The prothallium does not seem to be injured in the least by the presence of the fungus, and it is difficult to believe that any benefit is received from the endophyte. That the fungus receives food from the prothallium can scarcely be doubted.

Discussion

In comparison with the gametophyte of the Hepaticae, the prothallia of the Polypodiaceae and Osmundaceae seem to stand out conspicuously in two respects, namely, the longer duration of life,

and the capacity to produce plural sporophytes on the part of the gametophytes of the Hepaticae. In the Hepaticae as a rule the gametophyte is polycarpic, or capable of producing plural sporophytes; while in the Polypodiaceae especially, polyembryony is rare under conditions out of doors. Under experimental conditions, however, it has been demonstrated that the gametophyte may readily produce a number of sporophytes (MOTTIER 4).

Under ordinary conditions the gametophyte produces one sporophyte and dies; it is exhausted by the rapidly growing sporophyte. Prothallia of *Matteuccia nodulosa* may produce a sporophyte at the end of a month or six weeks, and when the plants are no larger than 2 mm. in width. This rapid "turn over" in the Polypodiaceae enables the self-nourishing prothallia to escape competition with more vigorous growths out of doors. Under cultural conditions growing prothallia for short periods of time is an easy task, but if prolonged throughout a period of years, the holding in check of more vigorous competitors requires incessant care. Prolonged life is possible only when exhaustion by sporophyte production is prevented, and when stronger competitors, such as mosses, etc., are kept in check.

As mentioned in a preceding paragraph, GOEBEL found that a prothallium of *Osmunda regalis* four years old had attained a length of 4 cm. In my cultures the prothallia of *O. claytoniana* easily attained a length of 3.5 cm. in fourteen months. This size is reached by the more vigorous plants if not crowded and if grown on soil. GOEBEL gave no data in regard to the method of cultivation. He seemed to be of the opinion that prothallia of the Filices would continue growth indefinitely if the production of sex organs could be prevented. In my cultures the production of antheridia and archegonia did not seem to impair the vigor of growth in any perceptible way. Throughout the entire time the prothallia, and their continuation shoots, produced sex organs continually. That which is necessary is the prevention of the production of sporophytes, in order that the prothallia may continue indefinitely. Referring further to the duration of life, GOEBEL (5) states:

Prothalli, upon which the act of fertilization has not been performed, may grow for a long time, but in them sooner or later phenomena of senescence appear, showing either in abnormal conformation or in the development of adventitious shoots.

In the light of the behavior of these cultures, I should not be inclined to look upon the formation of lateral proliferations or dichotomous branching as an indication of senescence, since such proliferations or shoots arise not only from the older proximal parts of the thallus, but from the growing ends as well. The continuation shoots resulting from one prothallium at the end of the fourth year, if not injured or too much crowded, continue to grow normally, showing the precise habit of one-year old plants. In *M. nodulosa*, for example, certain vigorous shoots of the four-year old cultures branch dichotomously and seem to be normal in every way. Some resemble closely the younger prothallia shown in figs. 1 and 2. Whether the habit of dichotomous branching is manifested in certain prothallia only, as an inherited character, or merely as an individual variation due to some external cause, cannot be stated with any degree of certainty.

The cells at the growing point of the four-year old shoots do not seem to differ in appearance structurally from those of younger plants; however, old age and decay result regularly in the older proximal tissues. At the end of the second year, and sometimes earlier, the proximal parts of the plants begin to turn brown, shrivel, dry out, and decay. From such older parts it very frequently happens that fresh proliferations develop, sometimes as larger shoots, sometimes as small irregular outgrowths replete with antheridia. Such outgrowths develop also from the younger parts of the thallus. It was observed frequently that fresh green proliferations sprang from the upper surface and edges of older proximal parts of midribs that were dark brown in color and apparently dead. Such older parts retain the power of developing local meristematic regions in a high degree. Sooner or later, however, the older parts gradually die, and this death progresses slowly in the direction of the growing point. Because of this fact the several branches become in time completely separated histologically. The rapidity of such death and decay is determined very largely by environic conditions. GOEBEL did not state whether his four-year old prothallium of *Osmunda regalis* 4 cm. in length had undergone any death or decay at the proximal end. Some of my plants attained a length of 3.5-4 cm. before the end of the second year, but after this time the proximal ends almost invariably began to die and shrivel up. Inasmuch as fresh shoots may

grow directly from the older tissues that have become brown on the surface, the interesting question arises as to what constitutes old age or senescence in such tissues.

As mentioned in a preceding paragraph, GOEBEL speaks of "abnormal conformation," or the development of adventitious shoots, as an indication of senescence in fern prothallia. In many cases abnormality or a marked departure of the ordinary form of the gametophytes resulted from what was looked upon as some external injury, or as the result of overcrowding. However, in both species studied certain departures in form and regularity of branching occurred from time to time in what seemed to be perfectly fresh and prolific plants, so that variations in form and the formation of shoots do not seem to indicate senescence.

One of the questions the writer hoped to answer through this study was as to how large a fern prothallium will become if not exhausted by the production of sporophytes or destroyed by some parasite or other external agency, if allowed to grow for a number of years. If no parts had died or decayed, a single prothallium of *Matteuccia nodulosa* and *Osmunda claytoniana*, in four years, under the cultural conditions described, and according to the development manifested in the cultures, would have covered a surface of 14,400 sq. mm. The largest continuous mass attained by one plant, without death of any part, is represented in fig. 11. Death and decay of old proximal portions set in near the close of the second year, and the surviving growth at the end of three and one-half years is shown in text fig. 7. The surviving growth of one plant of *Matteuccia nodulosa* is shown in text fig. 4. Many of the surviving shoots of these and other similar cultures are still growing as vigorously as younger prothallia, and unless they are destroyed by external agencies, there is no reason to doubt that they may continue indefinitely.

Summary

1. Prothallia of *Osmunda claytoniana* and *Matteuccia nodulosa* have been grown on soil for over three and one-half and four years respectively, under cultural conditions that prevented the production of sporophytes. When an occasional sporophyte appeared, it was amputated in such a manner as not to injure the gametophyte.

2. Prothallia thus grown branched both dichotomously and by the production of lateral shoots or proliferations. Proliferations, large and small, develop from the margins, from both surfaces of the midrib, and from the older proximal tissue, as well as from the growing point or apical sinus.

3. In older prothallia archegonia may be developed from both upper and lower surfaces. In such prothallia antheridia are borne chiefly on small marginal proliferations, or on small granular protuberances on older parts.

4. In some cases small protuberances are produced along the midrib upon the upper side. Such protuberances bear many archegonia.

5. The cell walls in older parts of the midrib are relatively thick, and are marked by numerous pits varying in size.

6. An endophytic fungus is present in certain circumscribed areas of older midribs of *Osmunda claytoniana*. The fungus has a tubular, branched, multinucleated mycelium. The hyphae may pass from cell to cell through the large pits in the cell walls. No fungal spores were observed.

7. A spinelike process developed from near the growing point in a few prothallia of *Matteuccia nodulosa*. These dried up as the prothallia grew older.

8. Apogamous sporophytes did not develop in any case.

9. If sporophytes are not produced, the continuation shoots of the prothallia seem to be able to continue growth indefinitely.

It gives me pleasure to acknowledge my indebtedness to my colleague, Dr. PAUL WEATHERWAX, for making the photographs used in the illustrations.

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EXPLANATION OF PLATE X

All figures were prepared from photographs. Figs. 1-8, *Matteuccia nodulosa*; figs. 9-11, *Osmunda claytoniana*.

FIGS. 1, 2.—Prothallia eight months old, showing dichotomous branching and typical ruffled character of wings and broad midrib; natural size.

FIG. 3.—Prothallium ten months old, showing more irregular mode of branching; $\times 1\frac{1}{2}$.

FIG. 4.—Prothallium ten months old which had not branched dichotomously; several proliferations have developed from older tissue of proximal end; $\times 1\frac{1}{2}$.

FIG. 5.—Prothallium which has branched irregularly; $\times 1\frac{1}{2}$.

FIG. 6.—Prothallium ten months old having six growing points resulting from dichotomous branching; at proximal end small granular-like protuberances bearing antheridia have developed; $\times 1\frac{1}{2}$.

FIG. 7.—Same plant as fig. 3, when one year old, showing irregular branching; wartlike protuberance at proximal (lower) end bears numerous antheridia; $\times 1\frac{1}{2}$.

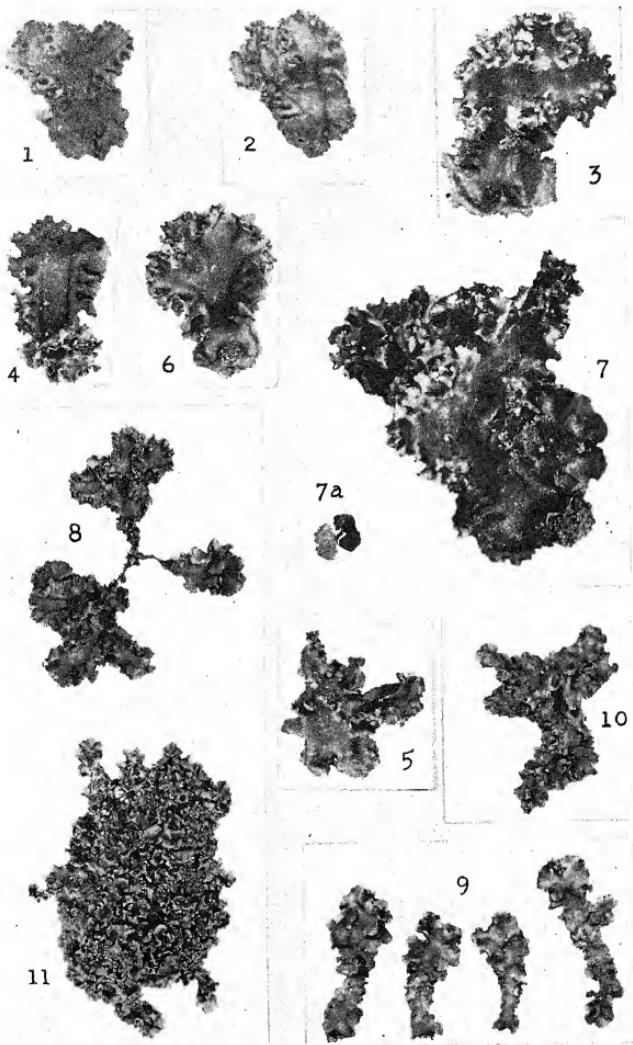
FIG. 7a.—Prothallium six weeks old, with sporophyte attached; $\times 1\frac{1}{2}$.

FIG. 8.—Prothallium two years and three months old, consisting of three large branches still connected by decaying midribs; branching regularly dichotomous; natural size.

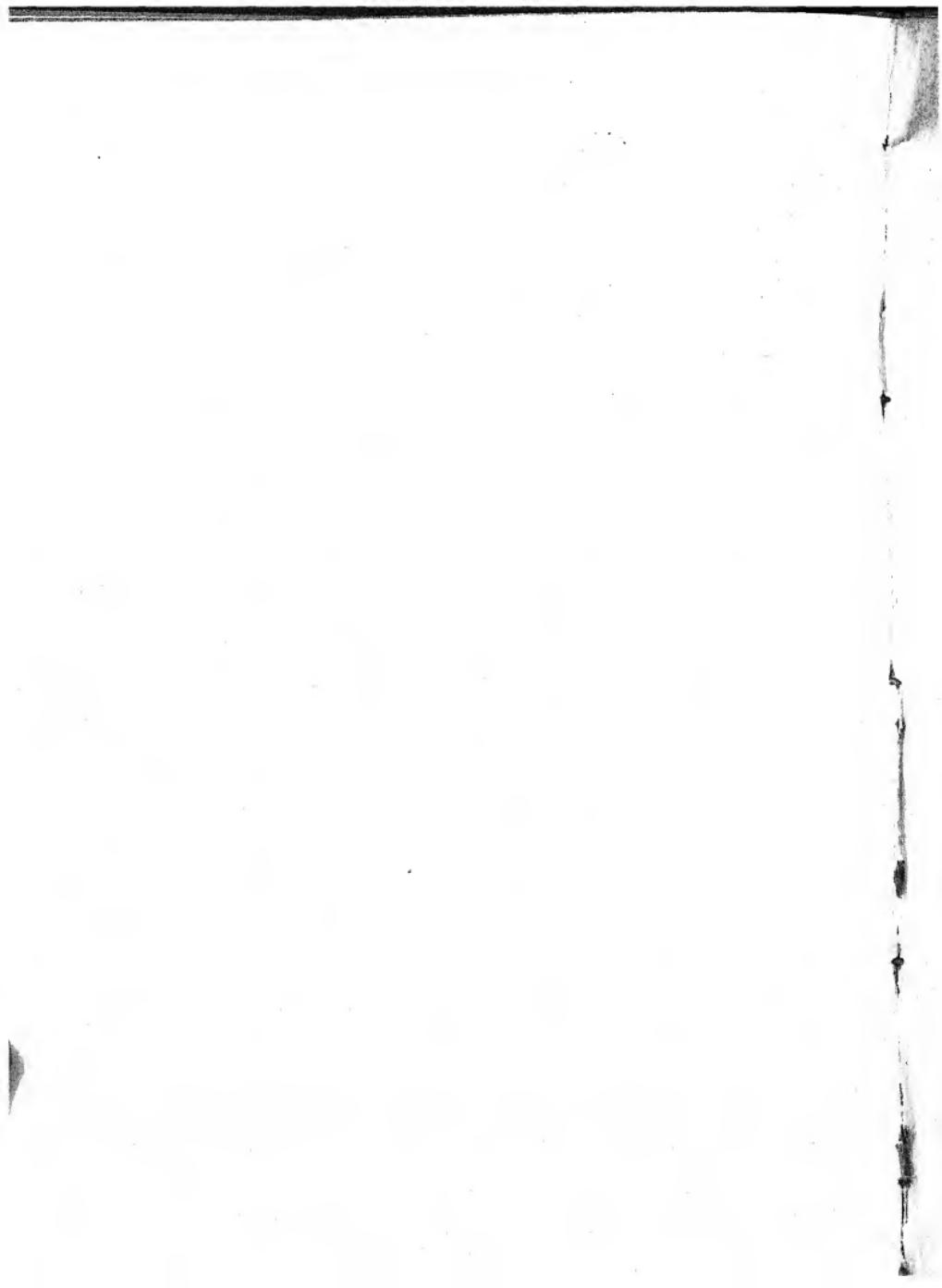
FIG. 9.—Four prothallia of *Osmunda claytoniana* from same culture, fourteen months old, showing growth typical in this species; natural size.

FIG. 10.—Prothallium twenty months old, somewhat irregularly branched; natural size.

FIG. 11.—Unusual prothallium of *O. claytoniana*, twenty months old; natural size.



MOTTIER on FERN PROTHALLIA



ECOLOGY OF FUNGI IN THE CHICAGO REGION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 367

V. O. GRAHAM

(WITH SIX FIGURES)

Introduction

Physiographic botany is concerned with the ecological relationships of the higher plants, and in this subject plants have been grouped into associations which in turn have been shown to belong to successions. The ecological relationships of fungi have been but little described, and an attempt is made in this paper to set forth some of the communities of fungi and their successional relations. The Agaricales and the larger Ascomycetes, the only fungi considered here, produce carpophores large enough to be readily observed in field study. The plan followed is to relate the fungi to the associations composed of the bryophyta, pteridophyta, and spermatophyta, which are considered in a general way in the successional order.

The physiographic features of the Chicago area are quite diversified. The moraine deposit with its depressions, the dune area and the adjoining swamps, the outcropping of Niagara limestone, the rivers, and Lake Michigan contribute to this diversity. Both the xerarch and the hydrarch successions are in evidence. These physiographic features are accompanied by plant associations of great variety. Related series of associations are found in the dune, bluff, swamp, bog, river, rock, and ravine successions. The successions of fungi that correspond to each of these are included in the study.

The difficulties attending the study of higher plants are very great unless situations involving a limited number of factors are chosen. An ecological study of fungi presents added difficulties because, unlike spermatophytes, fungi are readily identified only when in fruit, and in many cases the carpophores are in evidence for a short period of time only. Furthermore, the production of carpophores is affected by weather conditions to such an extent that many

species which normally occur in a certain habitat may be entirely absent throughout a season. This is true to a much less degree for the pteridophytes and spermatophytes. It is necessary, therefore, to accumulate, compare, and correlate evidence obtained through several years of observation in order to obtain a picture of a truly representative community of fungi. Eight years of observation have accumulated far too little evidence for conclusions concerning many of the successions discussed herein. The differentiating points for the identification of fungi are fewer, less definite, and more variable than for the spermatophytes. *Pleurotus ulmarius* illustrates their variability. Several standard descriptions have been made of this plant, none of which admits the scaly stemmed form so prevalent on the trees of our city streets. Forest forms have the smooth stem which corresponds to the standard descriptions. This is but one of the numerous examples in the taxonomy of fungi. The taxonomy must be mastered before the ecological study can be attempted. Figs. 1 and 2 present keys devised for the purpose of facilitating the taxonomic study. The peculiar nature of the differentiating characters of the carpophores makes such a key invaluable, especially to the beginner. Comparative sizes used in this discussion can be checked with fig. 2.

Seasonal occurrence

The flowering plants have a distinct seasonal sequence. Spring is represented by characteristic vernal species. The summer plants of the herbaceous type are of different aspect, and the autumn plants are larger than either the summer or the spring plants. The change of flora of the higher plants is paralleled by fungi, which present a different flora each season. *Morchella* and *Peziza* are representative of the spring, *Russula* and *Lactarius* of the summer months, and the autumn flora is represented by a tremendous number of species. Many which occur in the spring may be found throughout the summer, and some continue into the autumn; namely, *Collybia velutipes*, *C. dryophila*, *Pluteus cervinus*, and others. Scarcely any member of the genus *Morchella* is found at any other time than spring, and the same is true of *Peziza* with but a few exceptions. A greater number of forms represent the summer months, which are dominated by numerous species of *Russula* and *Lactarius*; these continue into

September, but are seldom found after October 1. Other summer genera represented to a considerable degree are *Amanita*, *Hygrophorus*, *Mycena*, *Omphalia*, *Psalliota*, and *Hypholoma*. *Tricholoma*, *Hebe-*

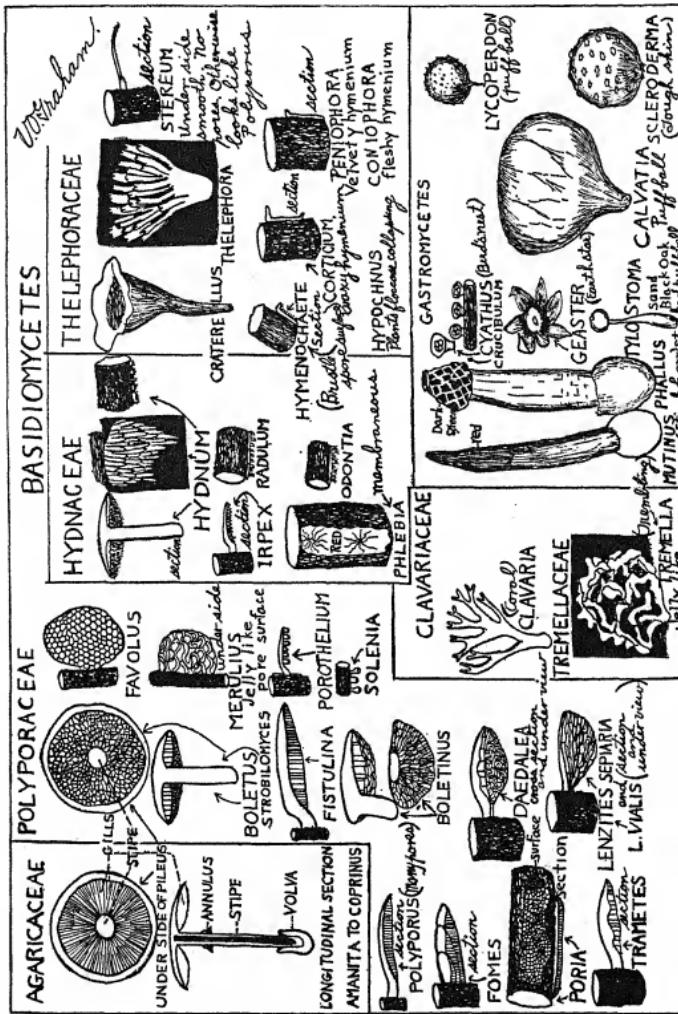


FIG. 1.—Diagram describing differentiating points of the divisions of the Basidiomycetes

loma, *Corinarius*, and other deep forest fungi are the dominating inhabitants of the autumn, while *Polyporus*, *Fomes*, *Trametes*, *Daedalea*, and *Lenzites* may be found in the winter. The winter spe-

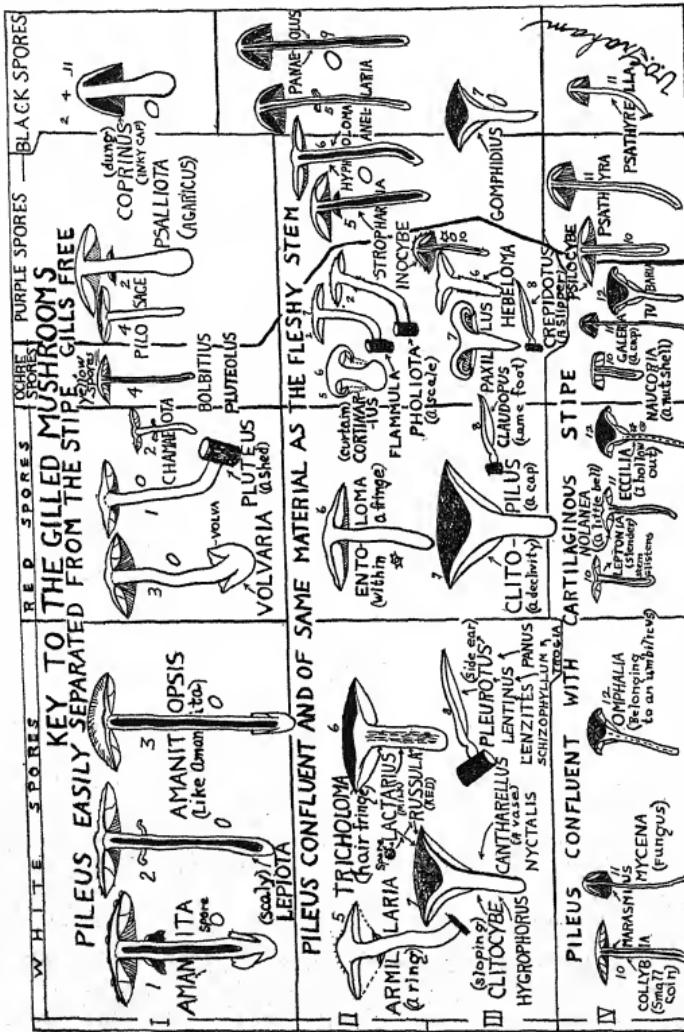


FIG. 2.—Key to the Agaricaceae, reading both vertically and horizontally

cies produce their carpophores in the warmer seasons and persist through the winter. *Collybia velutipes*, a soft species, produces carpophores at any season, but this is an exceptional case.

The abundance of fungi is also affected by the season. The spring and autumn species differ as to the type of habitat in which they are most abundant. In any habitat the humus supply is fairly constant, while soil temperature and moisture content are variable. Fungi are abundant in the shady forest in autumn but almost absent in spring, while in the pasture and open forest they are present in spring but absent in autumn. The pasture and sunny hillside, in an open forest, are abundantly supplied with moisture by the spring rains, but such a habitat is very dry in the summer and autumn. The shady forest floor with its leafy carpet retains moisture, but the retardation of the rise of temperature causes a paucity of fungi in spring and early summer. The ascent of soil temperature is greatly retarded in spring, while temperature decline is retarded in autumn; consequently fungi do not occur in a shady forest habitat until late in summer, but continue to occur until long after severe frosts in autumn. On the other hand, the soil of the pasture and other open habitats has an early rise of temperature, with moisture supplied by the spring rains, while the decomposing grass and dung furnish the necessary organic material. Such a habitat furnishes the majority of spring fungi. Due to the absence of moisture after the period of spring rains, the open grassy habitat is barren of fungi, but if rains are abundant throughout the summer, as sometimes happens, *Psalliota campestris*, *Galera tenera*, and species of *Panaeolus* will occur. The summer is more often very dry, hence xerophytic conditions prevail and fungi are absent. The presence of the three factors, sufficient humus, abundant moisture, and favorable temperature are necessary for the development of numerous carpophores.

The production of fungi is more retarded than the seasonal rise of temperature. The longest day of the year is June 21. Were it not for modifying factors this would be the hottest day, but retarding factors cause the season to be delayed and we conceive of the longest day of the year as the beginning rather than the middle of the summer. Because of the time required for growth, plants generally are retarded even more than the rise of the air temperature. Some flow-

ering plants because of their ability to store energy in their subterranean parts are able to grow quickly and bloom in the early spring. The growth process for fungi is much slower. We are accustomed to say "mushroom growth" when we refer to very quick growth, but the greater part, the mycelial development, is overlooked. Under most favorable conditions a rapidly growing species of *Coprinus* will develop carpophores in twelve days. Larger fungi may require months. The time consumed for the rise of the soil temperature, plus the time necessary for the growth of the mycelium, causes a great retardation of spring production of fungi.

Mycelial growth of less quantity is required to produce the smaller carpophores, but the time required for the production of the large carpophores is so great that they seldom occur in the spring. The spring species on the average are much smaller than the autumn ones, while those of the summer are of intermediate size. Fig. 2 shows the genera of fungi with cartilaginous stems to be much smaller than those of the other divisions. Such genera as *Marasmius*, *Mycena*, *Omphalia*, *Galera*, and *Collybia* are well represented in the spring, while *Russula*, *Lactarius*, *Amanita*, *Amanitopsis*, *Hygrophorus*, *Entoloma*, *Psalliota*, *Lepiota*, and *Panaeolus* are well represented in the summer. The larger species of these genera continue quite generally into September. This is a statement of a general observational conclusion and has many exceptions. *Tricholoma* is the most notable autumn genus in this region, but farther north *Cortinarius* becomes important. *Armillaria mellea*, so often attached to oak roots, is found in September and October in abundance; in November, after this species ceases to occur, *Tricholoma personatum* and *Hygrophorus Russula* push up their leafy covering as tufts on the forest floor. *Peziza coccinea* may be found in very late autumn and very early spring. Dr. W. B. McDougall says that it is likely that it requires a rather low soil temperature, perhaps a very cold soil with a somewhat warmer surface, a condition that usually exists in March and occasionally in December.

Trips in June to Palatine, where there is an open forest on rolling ground, revealed an interesting spring community. On the xerophytic moss *Ceratodon purpureum* occurs a community of *Omphalia fibu-*

loides. Sometimes a dozen carpophores are present on a patch of moss 3 ft. in diameter. The habitat of this moss is very dry in summer, but the abundant spring rains furnish moisture in the early season. Such is a typical spring community.

At Morton Grove in June appears another spring community in the grassy open forest. *Marasmius oreades*, and sometimes *Morchella esculenta*, are present in numbers where the open habitat makes possible the early rise of the soil temperature, and the spring rains have supplied the moisture. Either an old orchard or a south hillside exposure in an open forest is a favorable habitat for a community of *Morchella*. In autumn the north exposure is the better fungus habitat. The south exposure is usually grassy, the north is often moss covered; these, however, are not the determiners. The deficient spring factor is likely to be heat, and the south exposure receives the rays from the sun more vertically. The deficient factor in autumn is likely to be moisture. This is best retained where evaporation is least, and evaporation is least where the sun's rays are indirect or none. The south face is warmer but drier than the north face. The warm south face with its abundant moisture from the spring rains is a favorable habitat for vernal fungi. The north face with the accumulated heat retained in the soil by the vegetable covering is a favorable autumn habitat, but it is too cool in the spring for fungus growth.

Unshaded sand swales in the dune area are favorable for a community of fungi in the spring, with *Inocybe caesariata* one of the dominating species of this habitat. Due to the ever present supply of moisture, the sand swale is a favorable habitat throughout the summer and autumn.

In summary, the spring communities have the following characteristics: (1) the fungus community grows in the open; (2) carpophores are small; (3) the fungi grow on moss, in grass, or on dung; (4) the growth is in a habitat which is very dry during the summer and autumn.

The alternation of a dry and wet period makes possible the study of the ability of certain habitats to retain moisture and continue to produce fungi when other habitats cease production. During the dry

August of 1925 the ravine slopes continued to produce fungi for more than a week after other parts of the forest had ceased their productivity. The dry August followed a moist period in July. The accumulated moisture is to be found in the deeper soil layers during the dry period following the wet. The moisture moves upward by capillarity, and downward and horizontally by gravity. The horizontal movement causes the ravine slope to remain moist long after the flat forest floor has become quite dry; consequently the ravine continued to produce an abundant supply of fungi. In September, following the dry period, heavy rains fell. The forests were visited with the expectation of an abundant supply of fungi, but few were found. Not until the rains continued for three weeks did the growth of the carpophores become normal for the time of year. Close examination of the data revealed that the successive trips brought a harvest of fungi the size of which was successive. The smaller fungi were observed on the early trips, the larger on the later trips. It seems evident that the dry period was of sufficient severity to stop the growth of the mycelium. The return of the wet period found this growth entirely dormant. The energy in the mycelium had been spent in the continued production of carpophores after the beginning of the dry period. Time was consumed in the accumulation of energy for the renewal of carpophore production. The small species which appeared first belonged to the genera *Mycena*, *Marasmius*, and *Coprinus*. Later came the normal production of larger forms of *Russula*, *Lactarius*, and *Tricholoma*.

Due to low temperature, such habitats as dunes and swamps do not produce fungi in the spring. Cold air being heavier than warm, the soil of the swamp is colder than that of the hilltop. Furthermore, the water of the swamp warms more slowly than the land. These are retarding factors in the spring temperature rise of the swamp. Fungi are abundant here in the late summer and autumn, when sufficient time has elapsed for the rise of temperature. The snow falls on the sand in the dunes, and the moving sand carried forward by the wind covers it. More snow falls and in turn is sand-covered. The snow prevents the rise of the temperature of the sand, and the sand protects the snow from the sun. Moving sand habitats thus have a retarded rise of temperature.

Soil

A comparison of the soil of a floodplain with a bog, in which the factors are either similar or in contrast, proves interesting. Water is present in abundance in both cases; temperature seems to be equally favorable in both habitats; the differences will be found to be chiefly in the soil. The peat bog soil is made up of layer upon layer of sphagnum, so that vegetable material is abundant. An examination of this may be made at Cedar Lake, Lake Villa, Illinois. The material above the water is about 6 inches thick. The floodplain at River Forest is a soil rich in alluvium, a soil which would be given high rank for corn growing, especially if the possibility of flooding were removed. The humus of the floodplain is not abundant. The alluvial particles which make up this soil are deposited during high water, and the accumulation of humus continues for but a short time when deposition is repeated. The peat bog produces fungi abundantly in late summer and autumn, while the young floodplain is about as unproductive in fungi as are the bare sands of the dunes. Floodplains sufficiently elevated to be free from flooding, or old floodplains where humus has accumulated for many years, produce fungi as any other similar flat habitat. If the area is a forest it proceeds to the climax, and as such will have such fungi as are found in climax forests.

The soil of the forest floor has abundant humus, composed of decaying leaves and wood. The amount of humus present is comparable with the amount in the sphagnum bog. Both soils produce an abundance of fungi, but the fungus community on the one is very different from the community on the other soil. This indicates that the chemical nature of the humus is an important determiner. The effect of the different chemical nature of humus is also illustrated by the soil made up of accumulated white pine needles near Furnessville, Indiana, where the fungus flora is entirely different from that of the bog or the ordinary forest. In any of the habitats where the supply of humus is great the number of fungi is notable.

Aeration is important. When the supply of humus is constantly below water none of the larger basidiomycetes are present. It seems that such a place is not favorable for the development of the mycelium, but when the humus is raised above the surface of the water, as

it is at Cedar Lake, air may be present and the growth of mycelium is considerable. Here water is abundant but it does not interfere with aeration.

Successional associations

The bog succession may be studied at Cedar Lake and at Mineral Springs. It is convenient in the study of the communities of fungi to consider them in relation to the successional associations of the higher plants. The succession of the higher plants may roughly be separated into the following stages:

- | | |
|---|--|
| 1. <i>Decodon verticillatus</i> | 4. <i>Larix laricina</i> association |
| 2. <i>Sphagnum</i> , <i>Drosera</i> , <i>Sarracenia</i> ,
etc. | 5. <i>Betula lutea</i> — <i>Acer rubrum</i> associa-
tion |
| 3 Bog shrub association | 6. Climax association |

The first four of these associations are represented in the Cedar Lake study. This lake is a depression in the Valparaiso moraine deposit. Northwest of Chicago numerous depressions in the moraine constitute what may be spoken of in a general way as lakes of the Fox Lake region. Many tamarack bogs occur. Near the western shore of Cedar Lake occurs one of those rare physiographic situations known as a growing bog. The *Larix laricina* association here is composed of small trees, and no evidence can be found to indicate either the presence or the approach of the next association in the successional order, the *Acer rubrum*—*Betula lutea* association. *Typha latifolia*, *Carex hystericina*, and *C. comosa* are near the shore. Two hundred feet into the lake is a line of *Decodon verticillatus*, the outer fringe of the developing bog. On this first association may be found a small reddish *Peziza* with a ciliated margin, but no other fungi are present. The second association composed largely of sphagnum is quite extensive. The mat of sphagnum is very thick, readily supporting the weight of one's body. The mat is several inches thick and the water several feet deep. The surface quakes when walked upon. Other members of this association are *Drosera rotundifolia*, *Sarracenia purpurea*, and several mosses. The predominating members of the fungus community are *Galera hypnorum* and *Omphalia fibula*.

Another association has developed on the sphagnum, a shrub association composed of *Vaccinium macrocarpon*, *Betula pumila*, *Salix candida*, and *S. pedicellaris*. In this association is found *Hygro-*

phorus miniatus var. *sphagnophilus* as the dominating fungus species. It is also found in bogs throughout the region, wherever the association of sphagnum is at all extensive. Near Merrillville, Indiana, a bog has developed into the fourth stage, which is dominated by *Pinus Strobus* instead of *Larix laricina*. Here in August *H. miniatus* var. *sphagnophilus* is present. In the Cedar Lake bog it is present from August to November, gradually increasing in numbers until the first of October. The most striking fungus in this association is *Boletus spectabilis*. *Entoloma nidorosum* and *Hebeloma crustuliniforme* form *sphagnophilum* were also present.

The fourth association contains the tree *Larix laricina*. The shrubs and sphagnum form the undergrowth, and in fact the soil for the trees. It seems improbable that *Betula lutea* and *Acer rubrum* will ever enter here. Considerable time must elapse before the filling in will be sufficient for this to be a normal habitat for such trees. Civilization is encroaching upon the place, and may interfere with the natural course of events before the development of the habitat makes possible the entrance of the association of *Betula lutea* and *Acer rubrum*. It is quite difficult to be sure that any of the fungi here found may be separated from the sphagnum and the shrub association and placed exclusively in the *Larix* association. *Russula fallax* and a species of *Lactarius* were found under the *Larix*, which did not occur in the undergrowth where the *Larix* was not present. The following data were collected September 30, 1926:

ASSOCIATION OF HIGHER PLANTS	COMMUNITY OF FUNGI	NO. OF FUNGI
1. Decodon.....	Peziza.....	1
2. Sphagnum.....	Omphalia fibula.....	20
	Galera hypnorum.....	104
3. Shrubs on sphagnum.....	Entoloma nidorosum.....	20
	Hygrophorus miniatus var.....	200
	Boletus spectabilis.....	10
	Hebeloma crustuliniforme form sphag-	
	nophilum.....	8
4. Larix laricina.....	Russula fallax.....	3
	Lactarius sp.....	5

Fig. 3 shows the fungi found in the bog associations. These have their vegetative growth in the carpet of mosses, the prevailing one being sphagnum, but other mosses occur in sufficient abundance to

form a carpet somewhat independent of the sphagnum. On these moss carpets *Hygrophorus miniatus* occurs in great numbers.

The much older bog at Mineral Springs has the communities of fungi found in the older *Larix laricina* and *Betula lutea*—*Acer rubrum*

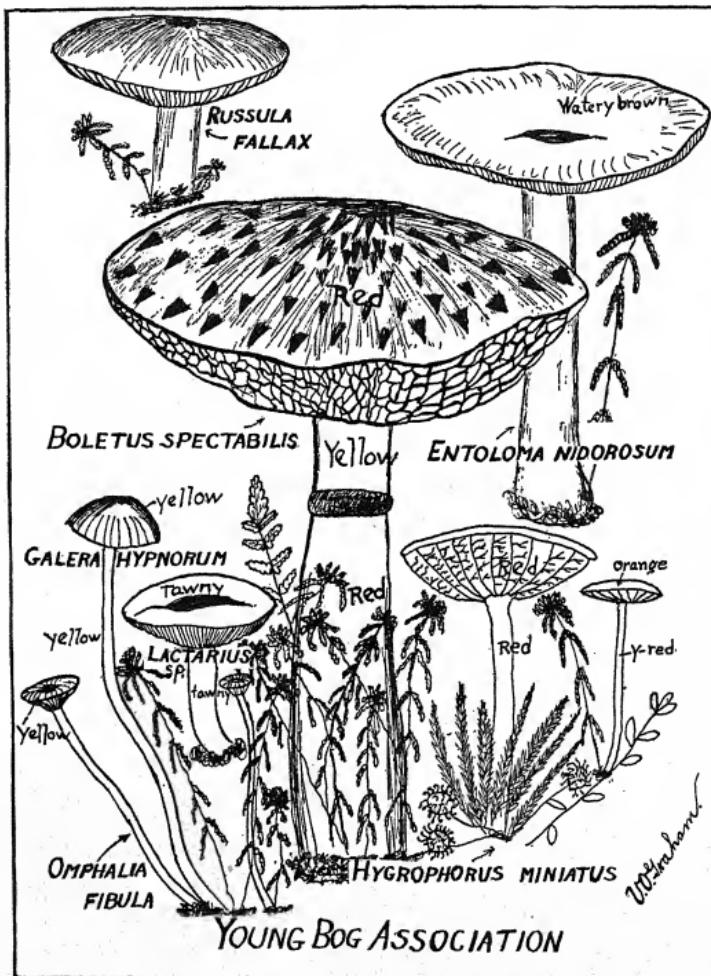


FIG. 3.—Young bog association, showing species of fungi most commonly found about October 1 on the floating land of Cedar Lake Bog at Lake Villa, Illinois.

associations. This bog is separated from Lake Michigan by dunes, its level being but little above that of the lake; consequently stagnant water is constantly present. Much care must be exercised here, as the habitats are adjacent to the xerarch succession of the dunes, and some overlapping of the fungus communities has occurred. The following is a somewhat uncertain list of the fungi found in the *Betula lutea*—*Acer rubrum* association: where *Larix* is present *Lactarius helvus*, *Cantharellus aurantiacus*, *Calvatia saccata*, *Hygrophorus speciosus*, and *Russula fallax* are found. These are also found in the association when *Larix* is absent, but in addition to them are *Collybia aquosa*, *Mycena sanguinolenta*, *Gomphidius maculatus*, *Russula delicata*, *Hypholoma lachrymabundum*, *Lactarius camphoratus*, *Hygrophorus chlorophanus*, and *Mycena epityrgia*. Much of the soil material of the association of *Larix* is certainly present in the soil of the succeeding associations; the continuance of species from former associations may therefore be expected. The presence in the beech forest at Tremont of *Hygrophorus chlorophanus* and *Cantharellus aurantiacus* indicates that the soil substance contains some of the same materials found in the *Betula lutea*—*Acer rubrum* association at Mineral Springs. This may point to a method of estimating the past successional history of this forest.

The beech forest at Tremont adjacent to the dunes has a mixture of the fungi that are to be expected in the xerarch situations mixed with the bog-hydrarch successional climax. These cannot readily be separated except as they have also occurred in a previous association in one or the other of the successions. There is no other habitat in this area with the climax association developed from the bog.

The river-hydrarch zonation of associations is well shown at River Forest. The succession of associations in this series appears roughly in the following order: water plants, amphibious plants, *Salix nigra*, *Acer saccharinum*, *Fraxinus americana*, *Ulmus americana*, *Quercus rubra*, and *Acer saccharum*. This is only a rough division for the purpose of comparison. The most conspicuous feature concerning fungi in this series is their absence. The question arises why fungi are not as abundant in the *Acer saccharinum* or the *Ulmus* association as they are in the *Acer rubrum* association of the bog series. The explanation is to be found in the absence in the one case

and the presence in the other of abundant humus, and in the exposure to floods in the one case and freedom from floods in the other. Two alluvial soil inhabitants may be found in the *Ulmus* association but are not common. These are *Lepiota alluvianus* and *Hypholoma velutinum*. The floodplain *Quercus rubra* association has a small number of fungi composed chiefly of those attached to buried débris. The fungus community is quite materially affected by the size of the river and the size of its watershed. The Desplaines River valley is often flooded. If this were not so the early associations of higher plants would be less extensive, the climax association would be proportionally greater in extent, humus would be in greater quantity, and fungi would be abundant. Such is the case at Edgebrook, where the north branch of the Chicago River has formed the floodplain. In the Edgebrook *Quercus rubra* association occurs *Lactarius vetus*, *Russula obscura*, *Thelephora Schweinitzii*, *Cortinarius coloratus*, and numerous epixyloous forms on débris and decaying stumps. In the forest at Olympia Fields the connection of the forest with the topography of the small stream flowing through it is more difficult to establish. The *Quercus rubra* association is very rich in fungi, and the association of fungi seems to belong to the ravine series. A comparison of the two hydrarch successions shows the following comparative points:

	BOG HYDRARCH	RIVER HYDRARCH
Humus supply.....	Abundant	Not abundant
Temperature.....	Favorable	Favorable
Moisture.....	Abundant	Abundant
Aeration.....	Good	Poor to medium
Alternation of flood and non-flood conditions.....	No	Yes
Removal of the accumulated humus.....	None or little	Much

The fen is represented in several localities in this area. Long Lake south of the dunes is bounded by fens sufficiently filled in for the growth of fungi. *Calamagrostis inexpansa* is the predominating grass. Humus composed of decaying grass and débris is present. A community composed of *Omphalia onisca*, *Clitocybe laccata*, *Pholiota mycenoides* (on moss), *Mutinus caninus* and on the débris *Pholiota unicolor* and *Pholiota marginata* is here present. Quite extensive fens

occur about half a mile from Lake Michigan north of Waukegan. As at Long Lake, no fungi occur where the water is constantly standing, but at the margin where *Calamagrostis inexpansa* occurs is the community with the species named. Another zone occurs here which has a community of *Hygrophorus miniatus*, *H. conicus*, *H. chlorophanus*, and *Leptonia asprella*. It is difficult to trace the succession any further in the Chicago area without an extensive study of the species found on the prairies. To make this study would require very careful observation, as the species on the prairie are doubtless few and far between. Fens have two possible developments: they may eventually develop into prairies or into the swamp forest.

The rock series is best represented near Lemont. By far the most important fungi in the early stages of this series are the lichens. These have been discussed by CALKINS and FINK. They occur in the rock succession in the order crustose, foliose, and fruticose. Such a habitat would not support fungi were it not for the peculiar structure of the lichen which makes nutrition possible by photosynthesis. Such species as *Placodium cinnabarinum* and *Lecanora hagenii* are common crustose lichens. *Physcia stellaris*, *Parmelia cetrata*, and *Dermatocarpon miniatum* are foliose lichens common on these rocks, while in the chinks and on ledges *Cladonia*, a fruticose lichen, is present. *Urnula craterium* occurs in the open forest of the rocky topography at Devils Lake, Wisconsin, and in the Illinois Ozarks. Other fungi found in this series in the succeeding associations are related to the accumulation of humus rather than to the underlying rock.

The dunes present a series of higher plant associations constituting a xerarch succession. Many of these are inhabited by fungus communities. The higher plant associations may be divided for this discussion into the middle beach with such annuals as *Cakile edentula*; the upper beach with the fore dune beginning its development around the grasses *Ammophila arenaria* and *Calamovilfa longifolia*; the *Pinus* association; the *Quercus velutina* association; the *Q. rubra* association; and the climax forest association. In the upper beach grass association is found a community of fungi composed of *Psilocybe arenulina* and *P. ammophila*. These have their vegetative growth in the sand, which seems to be barren of organic material so

far as can be determined by casual observation, but the presence of the fungi indicates that some organic material is present. By sliding the hand carefully below *Psilocybe arenulina* and lifting it, sand and all, it may then be gently lowered into a bucket of water. The mycelium floats outward, and its diameter may be about 3 inches. Sand particles cling to the hyphae. At least a film of organic material must be present on the sand particles. Near the roots of *Amorphila* and *Calamovilfa* may sometimes be found *Ithyphallus impudicus* and *Coprinus atramentarius*, and as these are generally associated with conditions created by man, their appearance here is doubtless subsequent to the advent of man.

The *Pinus* association is of three types: the young *Pinus* stage; *Pinus Banksiana*; and the very old *Pinus Strobus* association. The young *P. Strobus* association is inhabited by a community of fungi composed of *Clavaria muscoides*, *Clitocybe pinophila*, and *Lepiota cristata*. *Boletus americanus* sometimes enters this association, and occasionally *Lepiota cinnabarinus*.¹ The pine needle carpet becomes a mass of mycelium beneath the surface by the beginning of October. The surface needles alone appear to be unaffected by the enzymatic action of the mycelium. The action of fungi on the vegetable material of the forest floor breaks down the complex organic compounds into simpler ones. The soil by repeated deposition of leaves increases in humus supply. The young pine association contains but few species of fungi, but an old pine association such as occurs at the southern edge of the dunes at Furnessville, Indiana, contains many. The *Pinus Banksiana* association differs from the *P. Strobus* association in late October by the entrance of *Boletus granulatus*. The old associations of this tree contain many specimens of *B. americanus*, and occasionally *B. chrysenteron*.

The *Pinus Strobus* association at Furnessville, south of the dunes, contains trees measuring upward of 15 inches in diameter. The vegetable material on the soil is several times as thick as it is in the young pine association. The species of fungi may be more than a hundred, of which the dominating ones only are listed here. Very numerous on the forest floor, but usually overlooked because of small size, are

¹ This species is not listed by KAUFFMAN, but is well pictured in COOKE's volumes of colored plates.

Mycena vulgaris and *Marasmius androsaceus*. *Polyporus Schweinitzii*, shown in fig. 4, attaches to the pine roots. This figure also indicates the large size of the pine trees. Other species present in this association are *Clitocybe cyathiforme*, *Inocybe destricta*, *Tricholoma equestre*, *Craterellus dubius*, *Russula decolorans*, *Lactarius lignyotus*, *Hydnnum nigrum*, *Clitocybe catina*, *Tricholoma panaeolum* var. *caespitosum*.



FIG. 4.—*Polyporus Schweinitzii* in old pine association; size of trunk of *Pinus Strobus* in background indicates age of association; dark brown fungus grows attached to pine roots.

tosum, *Cortinarius semisanguineus*, *Russula chamaeleontina*, *Helvella crispa*, and *Clavaria flava*.

Coleybia butyracea, which occurs here also, appears in the *Juniperus virginiana* association of southern Ohio. It may be expected that plants dependent for nutrition on the organic material furnished by the higher plant association in which they occur, may sometimes occur in two associations, if these furnish organic material of similar chemical composition. This species continues to appear where the *Juniperus* has been replaced by *Quercus velutina*, indicating the persistence for a long time of the determining chemicals. The old pine association is the exception rather than the rule in this area. The

usual course of events initiates the *Quercus velutina* association without any great accumulation of humus under the pines. The usual association of fungi is therefore the one recorded for the young *Pinus* and the *P. Banksiana* associations.

The soil of the *Quercus velutina* association throughout the dunes, where changes are comparatively rapid, contains scanty humus. Areas become stabilized, but the protecting dune that made possible the stabilization may gradually shift, and the surface is again subject



FIG. 5.—*Amanita phalloides* in its habitat in the *Quercus velutina* association.

to wind action. The fungus community in this habitat composed of *Tylostoma campestris*, *Geaster hygrometricus*, *G. delicatus*, *Scleroderma flavidum*, and *Polyporus cinnamomeus* grows on scanty humus. The *Quercus velutina* association with considerable accumulation of humus is common in the protected areas, where leaves form a thin covering on the forest floor, so that the sand is not so bare as in the preceding habitat. The community consists of *Amanita phalloides* shown in fig. 5, *Hydnus zonatum*, *Hygrophorus virgineus*, *Tricholoma acre*, *T. transmutans* (KAUFFMAN states that this species forms mycorrhiza on the roots of *Quercus velutina*), *T. terriferum*, *Clitocybe ochropurpurea*, *T. personatum*, *Boletus affinis*, *B. felleus*, and *B. edulis*.

The next association of higher plants contains a fungus community composed of many species. The soil of the *Quercus rubra* association is carpeted with a leaf covering thick enough to supply organic material for the development of numerous fungi. Added to the favorable supply of humus is a favorable amount of moisture retained in the soil by the protective leaf covering. The soil, as previously explained, retains its temperature late into the autumn. The number of fungi when the season is rainy is tremendous. Among these may be enumerated *Omphalia gracillima*, *O. albidula*, *Clitocybe maxima*, *C. caespitosa*, *Entoloma jubatum*, *Hydnus aurantiacum*, *H. repandum*, *Cortinarius Atkinsonianus*, *Geaster rufescens*, and *Inocybe geophila*. In this same association, and occurring in protected ravines with the *Acer saccharum* association, are *Geaster triplex*, *G. saccatus*, *Clavaria cristata*, *Clitocybe candicans*, *C. claviceps*, *Amanita flavoconia*, *A. bisporiger*, *Russula virescens*, *Boletus separans*, *Psalliota abruptibulba*, *Hygrophorus flavodiscus*, *Lactarius insulsus*, *Russula mariae*, and *Hypholoma incertum* var. *sylvestris*.

The most common climax association of this area contains *Acer saccharum* as the predominating tree species. Such an association is nearly unmixed with other trees in the ravines at Lisle. Observations are not sufficiently complete for the entire separation of this fungus community from that found in the *Quercus rubra* association. *Clavaria pistillaris*, *C. fragilis*, *Lepiota cristatellus*, *L. rubrotincta*, *L. granulosa*, *L. glioderma*, and *Inocybe albodisca* belong to this ravine association. Doubtless the list should be much longer. Many fungi found in the climax forest are also found in preclimax associations. The explanation of this is to be found in the slow change of the humus material in the soil, and in the similarity of much of the leaf mould. Another consideration is the settling of the wind-carried leaves from the upland forests into the lower, protected, ravine forest. None of our *Acer saccharum* forests are extensive enough to prevent the mixing of the leaves on the forest floor.

The *Fagus grandifolia* climax forest at the eastern extremity of the area is not pure. Forests in other parts of the country indicate that *Boletus* furnishes a large number of species for this climax. Near Bainbridge, Ohio, in the ravines of the Rocky Fork canyons,

are beech forests almost unmixed with other trees. A two-hour visit here in late August, 1926, revealed a fungus flora of which nearly half of the individuals present were *Boleti*. Among these were *B. alveolatus*, *B. separans*, *B. subsanguineus*, *B. Russelli*, and others. It is stated by KAUFFMAN that certain *Boleti* form mycorrhiza on the roots of *Fagus*. This may indicate either a relationship between fungi and the advance of the forest to the climax, or the necessity

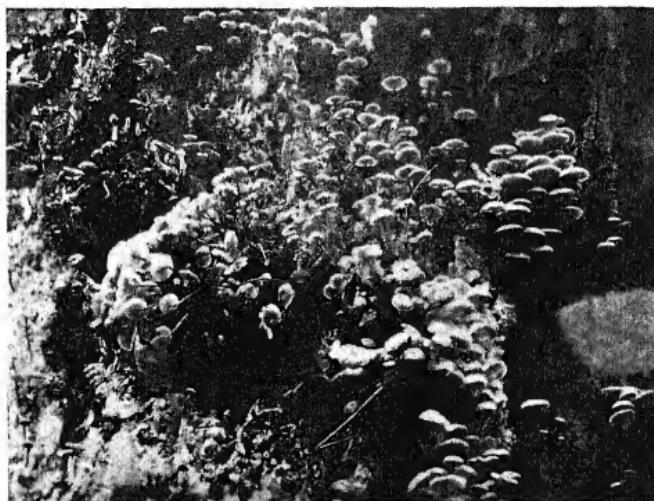


FIG. 6.—*Omphalia campanella* on a stump in the Olympia Fields forest, showing one of the species of fungi responsible for breaking down of complex to simple organic compounds, reduction of wood to humus.

of the presence of the *Fagus* rootlets for the development of the *Boleti*.

In the foregoing discussion, the terrestrial fungi only have been included in the associations. The chief interest of the epixyloous species within the scope of this paper is in that they reduce the woody material to soil humus. Fig. 6 shows a stump in the forest being reduced by enzymatic action of the fungus *Omphalia campanella*. Hundreds of epixyloous species are at work decaying stems, logs, and woody débris. A discussion of these should be contained in a separate article.

The nomenclature used for the higher plants is taken from Gray's *New manual of botany*, seventh edition. The nomenclature of the Agaricaceae is the same as that in *The Agaricaceae of Michigan*, by KAUFFMAN. The nomenclature of *Boletus* is that of PECK; The nomenclature of the Polyporaceae is the same as that contained in *The Polyporaceae of the middle-western United States*, by OVERHOLTS. In the Hydnaceae, Thelephoraceae, Clavariaceae, and Gastromycetes the nomenclature is the same as contained in *The higher fungi of the Chicago region*, by MOFFATT.

The writer is under obligation to Professor HENRY C. COWLES, of the University of Chicago, for help and inspiration in this work, and to Dr. W. B. McDougall, of the University of Illinois, for his critical examination of the material contained in this article.

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ORIGIN AND DEVELOPMENT OF TISSUES IN RHIZOME OF PTERIS AQUILINA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 368

C. Y. CHANG

(WITH EIGHTEEN FIGURES)

Introduction

The aim of the investigation here reported was to determine as accurately as possible the origin of tissues, and to trace their development to maturity. The need of anatomical studies of this nature the writer undertakes to demonstrate in the following pages.

The rhizome of *Pteris aquilina* was chosen for various reasons. It represents a class of stems that are creeping and subterranean, with peculiar problems of their own. The various structures of the stem stand out distinctly from one another, enabling one to trace them to their starting points with considerable certainty. The derivation of all cells, and consequently of all tissues from a single apical cell, brings the investigator closer to the common source of various tissues than is possible with a generalized meristem. Moreover, the availability of material in any desired quantity eliminates the danger of generalization on insufficient data.

The investigation deals first with the gross anatomy of the rhizome, then with the apical cell and its derivatives, the origin and development of endodermis, pericycle, phloem, and xylem, and finally the origin of adventitious roots.

Materials and methods

Stem tips of *Pteris aquilina* were collected in the sand dune region near Chicago. Collections were made mostly in June and early July, when the growth of the rhizome is most vigorous. As regards the field conditions of this most common fern, there is little to add to the extensive observations of HOFMEISTER (2), save the fact that the rhizome may be very deeply buried, sometimes 6-8 inches below the surface of the soil.

Gross anatomy

The internal structure of the rhizome is truly dorsiventral. A cross-section reveals the peripheral bundles on the lateral and ventral sides as small circles and ovals. Dorsally the peripheral bundle assumes the form of a band, frequently as long as the two central bundles. The two sclerenchyma bands are also decidedly different.

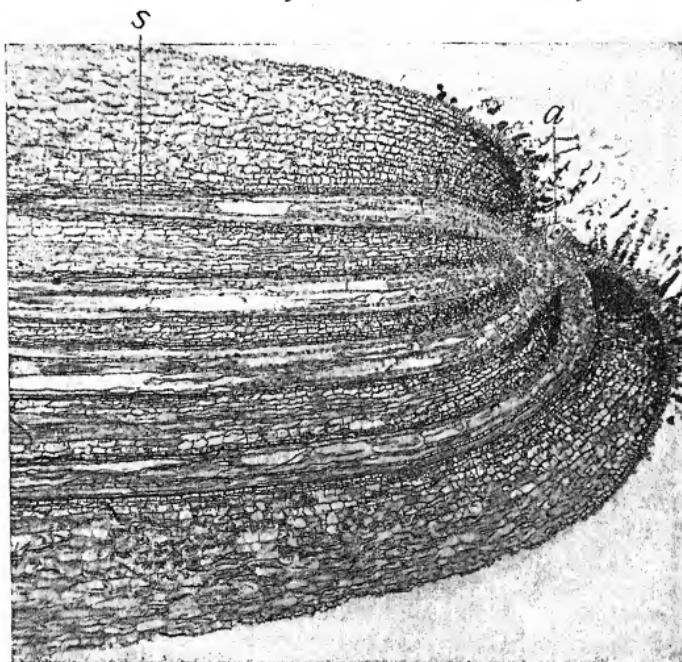


FIG. 1.—Longitudinal dorsiventral section of rhizome: *a*, apical cell; *s*, cells that will mature into sclerenchyma; X48.

The lower band is longer and curves upward, while the upper one is the shorter of the two, and usually straight. As a rule the lateral ridges are situated dorsally to the median plane. In making sections these features were utilized to orient the material imbedded in paraffin, and it was found that this dorsiventral arrangement held true in every case.

At the tip the bundles converge and meet behind the apex (fig. 1).

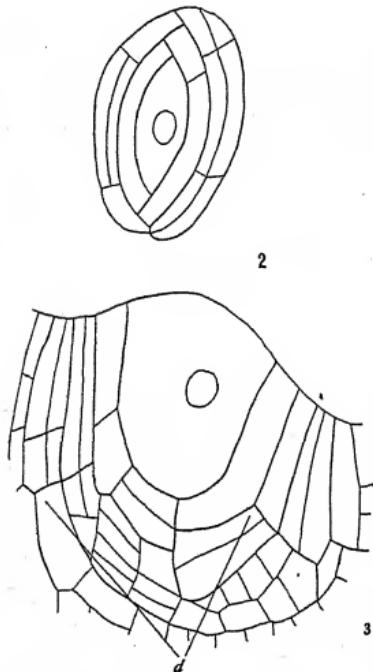
As the apical cell is situated dorsal to the median plane, the ventral peripheral bundles describe a large curve to come to the meeting point; hence at any given transverse plane of the rhizome, the ventral peripheral bundles are the oldest in the developmental history. The dorsal central bundle or the one above it (the dorsal peripheral bundle) may run practically straight to the apex, and consequently either may be the youngest in the development in a given transverse section.

The secondary roots are connected with the peripheral bundles on the ventral and lateral sides. The central bundles have no root connections.

Apical cell and derivatives

The apical cell of the stem, unlike that of the root, does not terminate the geometric axis of the stem, but is dorsally situated and turned upward (fig. 1a). The longest diameter of the apical cell makes an oblique angle with the axis of the stem. Of fourteen stem tips measured, this angle varies from $40\text{--}79^\circ$, the average being 62.5° .

The best view of the shape of the apical cell is to be obtained from transverse sections of the cell. To obtain this view the stem tip has to be mounted at such an angle that the microtome knife is most likely to strike the cell perpendicular to its longest diameter.



Figs. 2, 3.—Fig. 2, transverse section of apical cell; fig. 3, longitudinal section of apical cells; $\times 290$.

Since this diameter, as already pointed out, makes a varying angle with the geometric axis of the stem, sections perpendicular to it were obtained only after a large number of trials. In this view the apical

cell has the shape of a double convex lens, with the longer diameter parallel to the axis of the stem (fig. 2). It has two cutting faces, therefore, giving off concave-convex segments alternately right and left. As the apical cell with its immediate segments protrudes above the neighboring surface, the cross-section severs it from the adjacent tissues. The lateral view of this cell, a view parallel to the dorsoventral plane of the rhizome, shows it to be somewhat obovate in outline, with a broad curved free surface and a deep cup-shaped inner surface (fig. 3). It amounts to a modified form of the dolabrate apical cell that prevails in the anacrogynous Jungermanniaceae. Deviations approaching the triangular-pyramidal type occur.¹

Both anticlinal and periclinal divisions take place in the second segment of the apical cell. The outer half of the products of the periclinal division remains parenchymatous. It is from the inner half of the segment that desmogen strands arise (fig. 3 d). These are distinct from the surrounding tissue 8–10 cells below the apical cell (fig. 1). The tissue intervening between the peripheral and central bundles remains parenchymatous for a time, but 1 mm. below the apex most of its cells elongate and become prosenchymatous (fig. 1 s). Finally their cell walls become thickened and pitted, and the tissue matures into the sclerenchyma.

Five or six segments away from the apical cells, periclinal divisions of the peripheral cells suddenly increase, especially on the dorsal and lateral sides (fig. 1). As a result, an inclosure is built around the apex. The mode of formation of this pit is described at length by HOFMEISTER.

Endodermis

Long before the endodermis cells are filled with a dark staining substance, they are easily recognized by the Casparyan strips which line the radial and cross-walls of the endodermis cell (fig. 16 ca). These strips can be traced up the stem to within 0.7 cm. of the tip. In sections stained with safranin and light green the strip is recognizable at first as a green, more or less vacuolated band, but one or two cells down the green stain gradually gives place to the red, indicating the beginning of deposition of an impregnating material

¹ Cf. KLEIN (5), Pl. IX, figs. 23, 29, 30, 31.

which is regarded by PRIESTLEY and RADCLIFFE (8) as of a fatty acid nature.

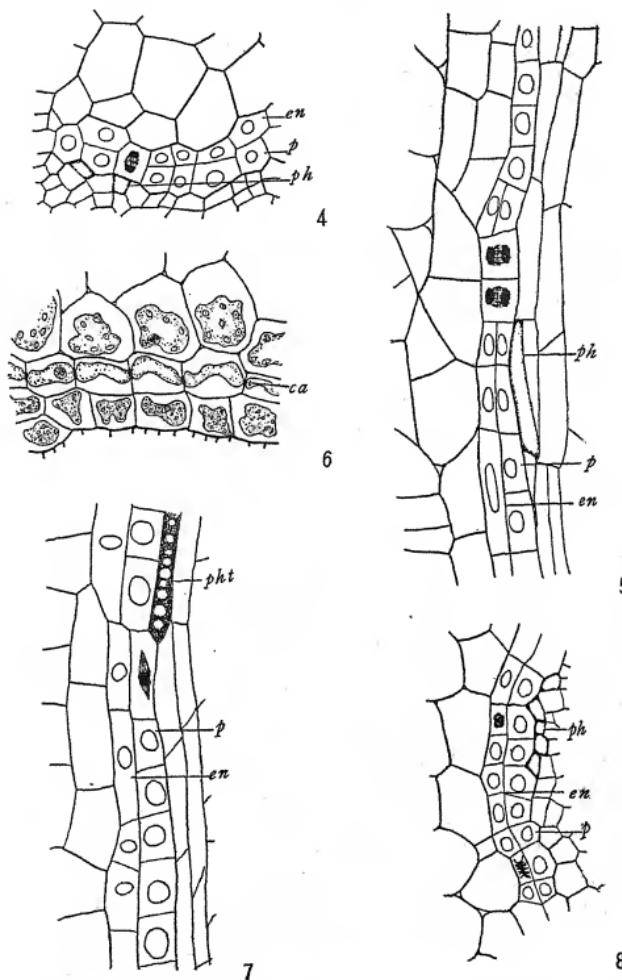
The endodermis and pericycle cells, which even in quite young tissue are readily distinguished by the shorter and denser cells of the latter, become indistinguishable in the embryonic region. This immediately suggests the probability that the cells of both layers are descendants of a common mother cell. Absolute proof of their common origin is not to be obtained, however, until mitotic figures are studied. These were observed in both longitudinal and transverse sections, and establish the fact that one cell divides periclinally, giving rise to the endodermis cell on the outside and pericycle cell on the inside (figs. 4, 5).

Although it is beyond the scope of the present paper to deal with the function of structures, it was noted that when the tissue underwent plasmolysis, the protoplast of the endodermis cell withdrew from all parts of the wall except from the Caspary ring. There the protoplast seemed to be "cemented" to the ring (fig. 6). This is in accordance with the claim of PRIESTLEY and NORTH (7) that water and solutes have to pass through the protoplast of the endodermis cell, and shows also that the semipermeable nature of the endodermis is not affected by plasmolysis. The air spaces between cortical cells are significant and will be discussed later.

Pericycle

The mature pericycle consists mainly of a single layer of cells, although in places doubled. As a rule these cells have a greater radial diameter than those of the endodermis. Beyond the meristematic region, where the cells of both layers are alike, the pericycle cells are shorter. This evidently is due to the fact that the pericycle cell has greater capacity for division and retains it longer. Sometimes the divisions are so frequent that the pericycle cells are isodiametric, while the endodermis cells are always elongate (fig. 7); but 0.5 cm. from the tip pericycle cells also cease dividing. The subsequent growth of the pericycle is entirely through elongation.

The origin of the pericycle has been treated in connection with that of the endodermis. The second layer of the pericycle, when there is any, ordinarily arises from the subsequent periclinal divi-



FIGS. 4-8.—Fig. 4, transverse section of rhizome, showing common origin of endodermis and pericycle (*en*, endodermis; *p*, pericycle; *pht*, protophloem sieve tube); fig. 5, longitudinal section showing same; fig. 6, transverse section through plasmolyzed tissue (*ca*, Caspary strip); fig. 7, longitudinal section showing relative length of endodermis and pericycle cells (*en*, endodermis; *p*, pericycle; *pht*, piece of protophloem sieve tube); fig. 8, transverse section a little later than fig. 4; $\times 430$.

sion of the first, but it was observed that the endodermal layer in the embryonic region may also divide once periclinally (fig. 8). As the mature endodermis observed is always uniseriate, it follows that the inner of the two daughter cells eventually becomes part of the pericycle, the outer one alone remaining endodermal.

The common origin of the endodermis and the pericycle naturally raises the question as to whether these two layers are cortical or stelar. If their common origin occurred very early, before the embryonic tissue differentiates into periblem and plerome, the question would always remain an open one, but such is not the case in the stem of *Pteris aquilina*. From the products of the apical cell plerome is very early distinguishable from periblem, by virtue of the denser contents and hence the deeper staining quality of the latter (fig. 1). The mother cell of the two layers is unmistakably on the stelar side. Further evidence is furnished by their kinship with cells of the protophloem. In both transverse and longitudinal sections, the mother cells of the endodermis and the pericycle on the one hand, and those of the protophloem on the other, were traced again to a common mother cell (figs. 9, 10); thus no room is left for doubt of the stelar origin of both the endodermis and the pericycle.

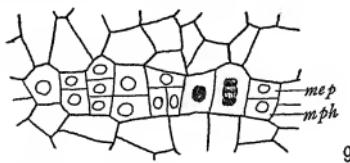
Phloem

PROTOPHLOEM.—Abutting on the pericycle on the inside is a uniseriate layer of protophloem sieve tubes, interrupted at frequent intervals by phloem parenchyma, and at the corners of large bundles often by xylem elements. In the cross-sectional view of the mature rhizome, the identity of these tiny tubes is obscured by the appearance of the parenchyma cells, which at this stage also possess thick walls and are often depleted of cell contents. In the developmental stage, however, the protophloem sieve tubes stand out from the thin walled, deep staining cells of the parenchyma.

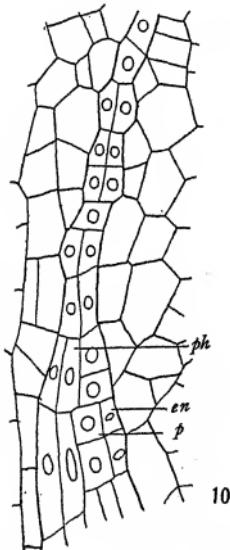
The thickenings on the wall of the protophloem sieve tubes appear early. In transverse sections 15μ thick the tubes begin to show secondary deposition on their walls at the fortieth section, or 0.6 mm. from the extreme tip,² when these cells are still dense with

² Descriptions here as in following cases are based on observations of large bundles with more or less straight courses. The differentiation of the lower peripheral bundles appears earlier in a given cross-section, as has been pointed out under Gross anatomy.

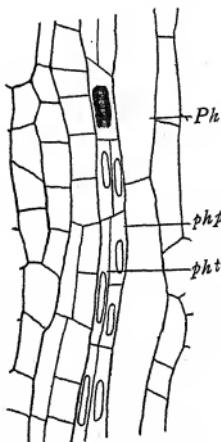
contents (figs. 4, 16). Above this point the cells of sieve tube destiny are indistinguishable from the neighboring phloem parenchyma cells.



9



10



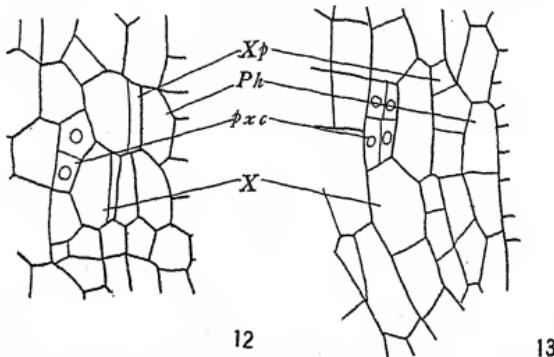
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FIGS. 9-11.—Fig. 9, transverse section showing common origin of endodermis, pericycle, and protophloem (*mep*, mother cell of endodermis and pericycle; *mph*, mother cell of protophloem); fig. 10, longitudinal section showing same feature (*en*, endodermis; *p*, pericycle; *ph*, protophloem); fig. 11, longitudinal section, showing common origin of protophloem sieve tubes and phloem parenchyma (*pht*, sieve tube cell of protophloem; *php*, parenchyma cell; *Ph*, metaphloem cell); $\times 430$.

In fact, as has been implied in connection with the description of the endodermis and pericycle, the sieve tubes and parenchyma cells are derived from a common layer of cells. A large number of cases was

observed where the mother cell was caught in the act of division, giving rise to two daughter cells, the outer one of which matures into a sieve tube, while the inner one remains parenchymatous (fig. 11). The common origin of the endodermis and pericycle and of the protophloem has already been described.

METAPHLOEM.—The metaphloem consists of sieve tubes only. In a number of cases the differentiation of metaphloem sieve tubes is scarcely behind that of the protophloem. At all events, the meta-



FIGS. 12, 13.—Fig. 12, transverse section 0.5 mm. from extreme tip (*pxc*, cells that produce protoxylem tracheids and parenchyma cells around them; *X*, metaxylem cell; *xp*, mother cell for a group of wood parenchyma cells; *Ph*, metaphloem cell); fig. 13, transverse section next to fig. 12; $\times 430$.

phloem develops remarkably early. Less than 0.5 mm. from the tip, a ring of cells inside of and next to the protophloem can be singled out as of metaphloem destiny by reason of their comparatively large caliber (figs. 12, 13 *Ph*), and as early as three or four cells below that, faint thickenings begin to appear as fine meshes on the walls (fig. 16). Localized sieve plates appear much later. Observations have led to the belief that these sieve areas are left as islands of thin places with tiny perforations, when the rest of the wall has been uniformly thickened with secondary deposition (fig. 16).

Xylem

PROTOXYLEM.—Each of the small bundles has one centrally placed protoxylem strand. The larger bundle as a rule has two

strands well spaced in the middle. Only when one bundle anastomoses with its neighbor may protoxylem be absent from it, although sometimes at some level a strand may consist of one or two elements only. Spiral thickenings begin to be laid down within 1 mm. of the tip. Traced from this point toward the apex, protoxylem cells can be seen as a group of elongate cells of small caliber, but here they cannot be distinguished from the surrounding cells of wood parenchyma. We have here a case parallel to that of protophloem development. The tracheids of protoxylem and parenchyma cells encircling it are close cousins. Their common ancestry can be traced to two cells (fig. 12), which divide into four (fig. 13). Irregular divisions then follow and several cells in the center of the group mature into tracheids (fig. 14). In development the protoxylem lags behind the protophloem in all bundles except the lower peripheral ones (fig. 15); there the protoxylem is a little ahead of the protophloem.

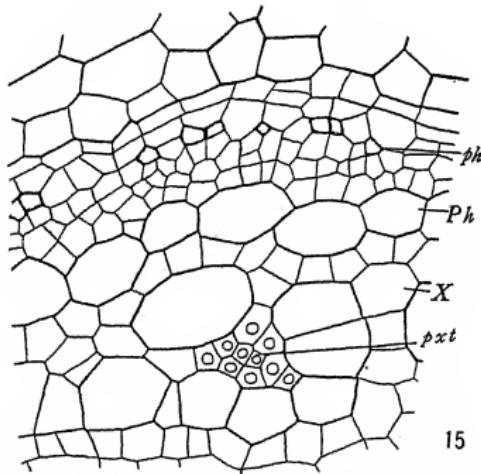
METAXYLEM.—Metaxylem cells have the curious feature of being the earliest group to differentiate and the last to mature. As early as twelve cells below the apical cell, those of metaxylem destiny are distinguishable by their larger lumina and rarefied contents, but as a rule the earliest secondary thickening cannot be detected until 0.5 cm. from this point (fig. 16). The wall thickenings of the metaxylem are laid down only after cell elongation has ceased.

Unlike the protoxylem cell, which acquires the initial cambiform shape through longitudinal divisions after the cessation of mitosis in the transverse plane, the metaxylem element gains its form entirely through elongation. Its mitotic activity ceases while still in the isodiametric form. It lengthens as its neighbors divide transversely, but the greatest elongation occurs with the general elongation of all cells, which in a vigorously growing rhizome takes place about 0.5 cm. from the tip. At this point the length of the cortical cells averages 120μ as compared with an average length of 14μ of the corresponding cells of the embryonic region. This is an increase of eight times. The metaxylem cell whose length subtends three or four cortical cells at the lower meristematic region is lengthened correspondingly. A general rule of cell elongation may be formulated. The cell which ceases division earliest in a given plane will be the longest cell in the direction perpendicular to that plane. For in-

stance, the cell which ceases division transversely two generations ahead of a cell at its right will be four times as long as the average of the granddaughter cells of its one time neighbor, whatever length



14



15

FIGS. 14, 15.—Fig. 14, transverse section showing protoxylem; fig. 15, transverse section showing earlier development of protophloem than protoxylem (*pht*, sieve tube of protophloem; *px*, protoxylem cells; *Ph*, metaphloem cell; *X*, metaxylem cell); $\times 430$.

the latter cells may attain. Exceptions must be made, of course, of cells haustorial in nature and of those which gain length by sliding past others. The cells that give rise to tracheids and sieve tubes cease to divide earlier in the transverse plane than the rest, and hence their greater length in the cell community.

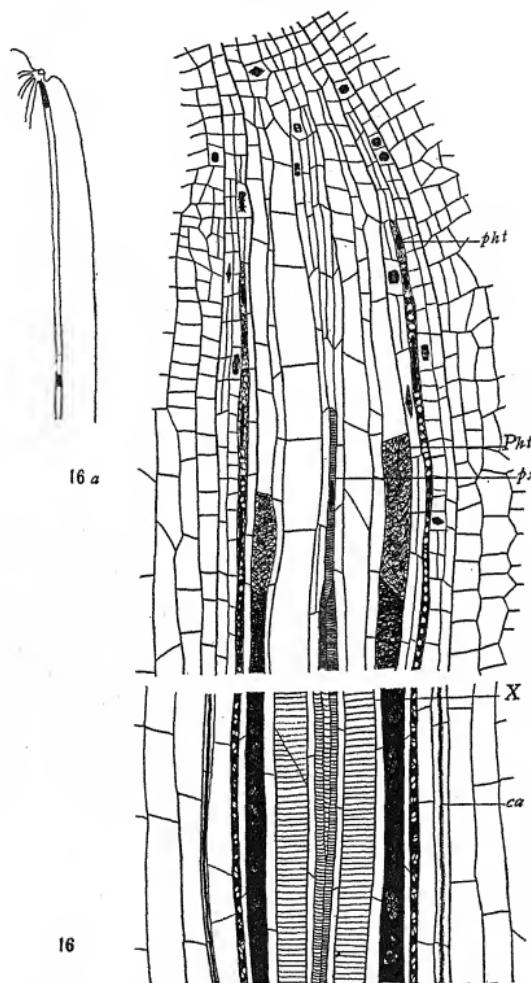


FIG. 16.—Reconstruction of bundle (*pht*, protophloem sieve tube; *Pht*, metaphloem sieve tube; *px*, protoxylem tracheid; *X*, tracheal segment of metaxylem; *ca*, Caspary strip of endodermis); hiatus represents an omission of 0.46 cm. of actual length, or 610 cm. on magnified scale; *a*, longitudinal section showing relative position of the two parts of fig. 16; $\times 134$.

WOOD PARENCHYMA.—The origin of wood parenchyma cells surrounding the protoxylem has already been described. The origin of the rest of wood parenchyma has not been thoroughly investigated. Cases were observed, however, which indicate that groups of parenchyma cells are descended each from a mother cell, and this cell is a sister of the neighboring metaxylem cell. Figs. 12 and 13, drawn from consecutive sections, show this mode of origin of cells of wood parenchyma clearly.

Adventitious root

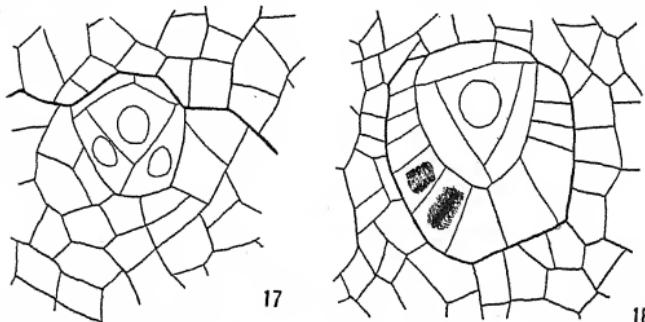
The adventitious roots appear extremely early. Cases were noted where a secondary root had reached a length of 1 cm. when it was only 2 mm. from the tip. The roots are connected with the small peripheral bundles on the ventral and lateral sides. When the small bundle begins to assume definite form, the large triangular-pyramidal apical cell of the root is already conspicuous with a number of segments cut off. It is clearly recognizable soon after the meristem is differentiated into periblem and plerome. This apical cell originates in the outermost layer of the plerome, but not infrequently the assignment requires careful study (fig. 17). It is obvious that the triangular-pyramidal cell is derived from an isodiametric one. The mode of producing a four-sided cell from a six-sided one is the same as in the Bryophytes. The outline of the original isodiametric cell generally remains distinct after a considerable number of subsequent divisions (fig. 18).

Discussion

The present findings on the shape of the apical cell of the rhizome are largely in accordance with the observations of KLEIN, so far as he has gone. Of the fifty species of ferns with creeping habit which KLEIN examined, he finds *Pteris aquilina* the only one that possesses an apical cell with two cutting faces "zweischneidig," the rest all having the triangular-pyramidal type. He concludes, therefore, that the form of the apical cell has no significance in relation to dorsiventrality. Still there are differences between *Pteris aquilina* on the one hand and the rest of the ferns with creeping stems on the other. To my knowledge, *Pteris* is the only one that is deeply penetrating; the other creeping forms are either trailing or very loosely covered with soil. Also *Pteris* is the only one with a dorsiventral structure. Thus

there is a striking coincidence (which may prove a correlation) between the "zweischneidig" apical cell and the truly dorsiventral rhizome, a difference which should not be ignored.

In his observations the writer has found nothing that is out of harmony with the opinion of LAND³ that the apical arrangement of *Pteris* is a case of admirable adaptation. The growing point is turned upward, presenting the older and firmer tissue to the pressure of the forward thrust. It is located at the bottom of a pit, which protects it from any abrasion as the rhizome elongates, and the delicate point



FIGS. 17, 18.—Fig. 17, section parallel to transverse view of apical cell of rhizome, showing apical cell of root just after root formation; heavy line marks boundary between periblem and plerome; fig. 18, later view of apical cell of root; $\times 430$.

is further protected by a very dense covering of hairy scales. This opinion is strengthened by the fact that in *Polypodium aureum* and *P. vulgare*, which are creeping but superficial, the apical cell terminates the geometric axis of the stem.

In his work on the anatomy of the leaves and stems of ferns, Russow (9) observed the similarity in shape and size of the juvenile cells of the endodermis and the pericycle, and concluded that there was a common origin for these two layers in *Pteris aquilina* and other forms. The unreliability of conclusions drawn from the appearance of cell shape, however, is illustrated by the fact that VAN TIEGHEM and DOULIOT (13) in their work claim a separate origin for these two layers in *Pteris*. That no importance has been attached to Russow's

³ Private communication.

report was shown by a statement of JEFFREY (4), who, in describing the bundle of *Pteris aquilina*, states:

Within the endodermis is situated a layer one or sometimes two cells in breadth, the pericycle, which constitutes the external boundary of the fibro-vascular tissues, just as the endodermis marks the internal limit of the fundamental system.

The similarity in appearance of cell shape that led Russow to believe in the common origin of the endodermis and pericycle, also led him to conclude that both these layers were derived from the ground tissue, a conclusion which the writer's findings contradict.

The astonishing fact, however, is that while the statement that the endodermis constitutes the innermost layer of cortex is found almost universally in botanical textbooks, no conclusive account based on actual observation has been found, which shows this mode of origin of the endodermis. VAN TIEGHEM's positive statements were not supported by illustrations (12), and were contradicted by reports of his contemporaries (3, 6). Concerning the figures by VAN TIEGHEM and DOULIOT (13), a number of which support VAN TIEGHEM's claim, SCHOUTE (10) states:

Die gegebenen Figuren, von VAN TIEGHEM und DOULIOT nach aus freier Hand angefertigten Schnitten gezeichnet, sind aber sehr schematisch.

The comparatively recent work on the origin of the endodermis was on *Hippuris*, investigated by SCHOUTE (10), and later confirmed by BARRATT (1). They found that the endodermis and several layers outside of it were derived from the plerome.

If with further investigation it proves to be generally true that the endodermis is a part of the stele, then any theory on structural evolution which bases the main weight of its argument on the endodermis being cortical will necessarily require reconsideration.

The common origin of protophloem sieve tubes and phloem parenchyma makes the latter a part of protophloem. The fact that from a homogeneous group of cells, only the outer ring is transformed into sieve tubes, is probably not without significance. A parallel case is the development of endodermis and pericycle. Starting from two

sister cells exactly alike in all respects but position, the endodermis cell undergoes pronounced transformation through acquiring a Casparyan ring, and later a suberized wall (8), while the pericycle cell remains unchanged. A still more remarkable fact is that when a juvenile endodermis cell divides tangentially, the inner one of the two cells remains simple and eventually forms a part of the pericycle; the outer one alone acquires the characteristics of an endodermis cell. It will be remembered that the cortex is filled with air spaces up to the endodermis (fig. 7). PRIESTLEY and NORTH point out that suberin formation is always dependent upon the presence of oxygen. It is the position with respect to oxygen supply, therefore, that determines the formation of structures characteristic of the endodermis cell. It is not without reason that one expects that the differentiation of the outer layer of protophloem into sieve tubes may also be due to its relative position, although the determining factor is not yet known.

The comparatively greater age of phloem in *Pteris* rhizome does not agree with the findings of TURNER (11) in the stem of *Lycopodium lucidulum*. In that form TURNER finds that the development of xylem is ahead of phloem. This apparent disparity is readily explained, however, when one compares the habit of the two stems: the stem of *Lycopodium lucidulum* is erect and aerial, while that of *Pteris aquilina* is creeping and subterranean. The developing stem of the former is right in the photosynthetic region, but away from water and mineral nutrients, whereas exactly the reverse condition prevails at the growing tip of *Pteris* rhizome. Water transportation is more active in one, food translocation in the other. The structural development corresponds admirably with these physiological conditions.

The comparative earliness of protoxylem development in the lower peripheral bundles of *Pteris* rhizome is probably due to their communication with the adventitious roots. A close connection with an absorptive organ is expected to have a stimulating effect on the development of a conductive structure.

Concerning the origin of the adventitious root, VAN TIEGHEM and DOULIOT came to the following conclusion:

En résumé, que la tige des Fougères, soit monostelique ou polystelique, . . . la racine latérale s'y fait toujours aux dépens d'une cellule de l'endoderme actuel.

Thus these authors contrast the origin of the secondary root of ferns with that of seed plants, in which they found the source of adventitious roots to be the pericycle. The present investigation shows that the secondary root of *Pteris* starts so early that it is impossible to assign it to any morphological layer, but as it originates in a cell in the outermost layer of the plerome, it cannot help involving the endodermis, which, as has been shown, is the limiting layer of the stele. In fact, when VAN TIEGHEM and DOULIOT say that the root originates in an endodermis cell, they mean in a cell among the products of which arises the endodermis. There is, however, this radical difference. VAN TIEGHEM and DOULIOT claim that the initial of the root arises from the innermost layer of the cortex, which they consider to be the seat of the endodermis, whereas the writer's preparations show that both the initial of the root and the mother cell of the endodermis originate in the outermost layer of the stele. Both are stelar, not cortical. To show the origin of secondary roots in ferns the authors in question figured the stem of *Nephrolepis davallioides* only, and one hesitates to accept their statement since they showed the apical cell only in the advanced stage, when its assignment to any histogenic region is largely a matter of conjecture.

In conclusion, it is considered that the results of the present investigation warrant the opinion that it is only through critical study of the embryonic region that we can determine with certainty the origin of tissues, and that work which was done long ago may be worth reinvestigation.

Summary

1. The structure of *Pteris* rhizome is unmistakably and invariably dorsiventral.
2. In shape the apical cell of the rhizome is a modified form of the dolabrate type.
3. The apical arrangement is considered to be adaptive to the subterranean habit.
4. The endodermis and pericycle have a common origin and both layers are stelar.

5. The protophloem sieve tubes and the phloem parenchyma are derived from a common mother cell, which in turn has a common origin with the mother cell of the endodermis and the pericycle.

6. Protoxylem, metaxylem, wood parenchyma, and metaphloem were traced also, so far as the identity of their cells was recognizable.

7. The development of xylem lags behind that of phloem in all except the lower peripheral bundles. This difference in development is considered to be due to the subterranean habit of the rhizome.

8. The adventitious root develops extremely early. Its apical cell originates in the outermost layer of the plerome soon after its differentiation from the periblem.

9. The results of the present investigation on the origin of the endodermis and adventitious root do not agree with the descriptions in the older works.

The writer takes great pleasure in acknowledging his indebtedness to Professor W. J. G. LAND, under whose direction this investigation has been conducted. Thanks are also due to Professor C. J. CHAMBERLAIN for his kindness and encouragement.

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RELATION BETWEEN FRUIT SIZE AND ABSCISSION OF YOUNG ORANGE FRUITS¹

A. R. C. HAAS

Excessive abscission of young citrus fruits during certain periods of the year constitutes a serious loss to the citrus industry. CORR and HODGSON² have described the conditions under which the navel orange crop of 1917 in certain districts became practically a total loss. Among the factors considered, particular stress was placed upon the high maximum daily temperatures that accompanied dry winds. Citrus leaves are able to withdraw moisture from the fruit, and the daily water deficits brought about in this way may cause abscission, or may interfere greatly with the development of the fruit.

The present paper is concerned with the so-called "June drop" of young citrus fruits under high maximum daily temperature, and recognizes the fact that during such periods there may be a large withdrawal of moisture from the fruit by excessive leaf evaporation. The data presented here have particular reference to excessive abscission of young fruits from citrus trees that receive the best cultural care known, and the discussion has no intended application to abscission of young citrus fruits brought about by deficient nitrogen supply, unfavorable soil salinity, deficient soil moisture, etc., conditions under which abscission of the fruits is a reasonable expectation.

It has been well established³ that citrus leaves are able to withdraw moisture from the fruit. The writer has found in a large series of unreported experiments that the upper surface of mature citrus

¹ Paper no. 156, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² CORR, J. E., and HODGSON, R. W., An investigation of the abnormal shedding of young fruits of the Washington navel orange. *Univ. Calif. Publ. Agric. Sci.* 3:283. 1919.

³ BARTHOLOMEW, E. T., Internal decline of lemons. III. Water deficit in lemon fruits caused by excessive leaf evaporation. *Amer. Jour. Bot.* 13:102. 1926.

leaves is practically no more economical of the moisture of the leaf than is the lower surface, and that mature citrus leaves are much less economical of their moisture than are young ones, but that in contrast the young leaves of the walnut are much less economical of their moisture than are mature ones. The almost complete absence of stomatal regulation, and the large cuticular evaporation from both surfaces of mature citrus leaves, bring about water deficits in citrus foliage and fruits during periods in which high maximum temperatures prevail, and especially when such temperatures are accompanied by dry winds. The injurious effect of such water deficits may not be evident unless their action is severe and prolonged. It is rather difficult to evaluate the effect of excessive leaf evaporation upon abscission of citrus fruits, unless we also consider the evaporation from the fruit itself. Heretofore the large evaporating power of citrus leaves and their consequent withdrawal of moisture from the fruits have caused the evaporation from the fruits to be practically ignored, although in fact there is a great difference in surface evaporation between young and older fruits.

During the month of July, 1925, at the Rubidoux Tract of the Citrus Experiment Station, high maximum temperatures were reached quickly and were maintained for but very short periods. The writer was conducting large tank experiments with Valencia orange trees in soil cultures at the time, and noted the excessive shedding of young fruits of a certain size, whereas fruits of larger sizes remained firmly attached until maturity. The soil in the tanks had been kept uniformly moist during the hot weather, and the trees were protected by a solid lath windbreak from hot, drying winds. Examination of orange trees in the experimental plots of the Station also revealed excessive abscission of the smaller citrus fruits under favorable soil moisture conditions.

Young navel oranges were picked from guard rows at the Rubidoux plots, and were immediately brought into the laboratory. The fruits were air dried in a room at about 80° F. The effect of the size of the fruit upon the loss of moisture from its surface is seen in table I. The water loss is stated as the percentage of total water lost on drying to constant weight at 140°-150° F.

Table I shows that the young fruits differ considerably in their

loss of moisture, according to their size. The smaller the fruits the more rapid is the water loss, under a given set of conditions.

In a repetition of this experiment, the fruits were dried at 80° F. in desiccators containing anhydrous calcium chloride. Several large

TABLE I
PERCENTAGE WATER LOSS FROM FRUITS OF WASHINGTON
NAVEL ORANGE, AIR DRIED FOR TIME INDICATED

TIME (HOURS AND MINUTES)	WATER LOSS	
	Fruits (65), diameter about 1.7 cm.	Fruits (58), diameter about 2.5 cm.
25: 0.....	21.38	13.93
44:30.....	29.56	19.67
71:30.....	42.14	27.87
93:10.....	49.69	33.60
139: 5.....	61.01	42.95
162:35.....	66.66	48.36
188: 5.....	71.70	53.44

desiccators were used, and a proportionate number of fruits of each size was placed in each desiccator. In every case the fruits were left standing on the stem end.

TABLE II
PERCENTAGE WATER LOSS FROM FRUITS OF WASHINGTON
NAVEL ORANGE, AIR DRIED FOR TIME INDICATED

TIME (HOURS AND MINUTES)	WATER LOSS	
	Fruits (63), diameter about 15×20 mm.	Fruits (40), diameter about 35×40 mm.
23:30.....	6.30	2.31
66:30.....	14.96	6.29
93:25.....	18.89	8.17
114:15.....	21.26	10.07
161: 0.....	25.98	12.58
184:25.....	29.13	14.26
210: 0.....	31.50	16.14

Table II shows that young citrus fruits may lose their moisture two to three times as rapidly as larger fruits. Another lot of 63 navel oranges (diameter 15 mm. × 20 mm.) in desiccators lost 7.37 per cent of their moisture in 24 hours, while 40 navel oranges (diameter 35 mm. × 40 mm.) lost 2.15 per cent in the same period. The rind of the

smaller fruits was about 5-6 mm. in thickness, while that of the larger fruits was about 6-7 mm.

Eighty of the small navel oranges and 33 of the larger ones were dried in an oven which was regulated to 110° F., but which rose even above 120° F. once the fruit was placed in it. The smaller oranges lost 65.61 per cent of their moisture and the larger ones 54.51 per cent in 23 hours and 50 minutes. The effect of the higher drying temperatures appears to reduce somewhat the differences in the percentages of moisture lost by the oranges of different sizes.

It was of interest to find, in the case of grapefruit also, that the smaller fruits lost a considerably higher percentage of their moisture when dried at 70-80° F. than did larger fruits (table III).

TABLE III
PERCENTAGE WATER LOSS FROM GRAPEFRUIT AIR
DRIED FOR TIME INDICATED

TIME (HOURS AND MINUTES)	WATER LOSS	
	Grapefruit (93), diameter 25×30 mm.	Grapefruit (42), diameter 40-45×45-50 mm.
19:25.....	16.73	9.42
44: 0.....	30.15	19.11
67:35.....	41.05	27.24

The water losses from grapefruit and Valencia oranges of approximately similar size were compared, to ascertain whether grapefruit loses its moisture less readily than Valencia oranges, and thereby is enabled to remain attached in groups or clusters even to maturity. The data in table IV show that young grapefruits lose their moisture at about the same rate as young Valencia oranges of similar size.

A field test was made to determine whether the effect of high temperatures, when accompanied by a favorable soil moisture supply, decreases with an increase in the size of citrus fruits. Young Valencia oranges (diameter 18 mm. × 20 mm.) were tagged on trees in the guard rows of the Rubidoux plots on June 29, 1925. On July 22, only 36 out of 113 tagged fruit still remained attached, while among the tagged fruits of slightly larger size there was practically no abscission.

The water content of large citrus fruits is proportionately not much different from that of small fruits. For example, in table I the water content of the small fruits was 74.6 per cent of the fresh weight, while that of the large fruits was 76.2 per cent; in table III the water contents are 80.4 and 80.5 per cent respectively. Consequently the greater percentage loss of water from the small than from the large orange fruits is not due to differences in the water-holding capacity of the fruits of different sizes. The possibility exists that the reduced loss of water from the large fruit may be due to

TABLE IV
COMPARATIVE PERCENTAGE WATER LOSS OF SIMILAR SIZED YOUNG GRAPEFRUITS
AND VALENCIA ORANGES

TIME (HOURS AND MINUTES)	WATER LOSS IN AIR DRYING AT 70°-80° F.		WATER LOSS IN AIR DRYING AT 102°-106° F.	
	Grapefruit (70), diameter 18×21 mm.	Valencia oranges (58), diameter 18×21 mm.	Grapefruit (70), diameter 18×21 mm.	Valencia oranges (58), diameter 18×21 mm.
23:25.....	22.31	19.03	45.04	46.29
46:20.....	36.36	31.86	69.01	71.61

deposits of gums or resins in stomatal openings. The stomatal openings of small and large navel orange fruits were eliminated by peeling off the outer green portion of the rind. Under uniform exposure on trays of large mesh wire screen, the small fruits lost 48 per cent of their water in 23 hours, while the large fruits lost only 24 per cent in the same period. Obviously the greater rate of water loss from young fruits is not due to an appreciable extent to any surface mechanism that controls the loss of water through the rind. The results may be explained on the basis of the relation of the surface area of the fruit to its volume. If for the sake of convenience we assume that the shape of an orange is that of a sphere, we have to deal with the following formulae:

$$\text{area of a spherical surface} = 4\pi r^2 \quad (1)$$

$$\text{volume of a sphere} = \frac{4}{3}\pi r^3 \quad (2)$$

$$\frac{\text{surface area}}{\text{volume}} = \frac{4\pi r^2}{\frac{4}{3}\pi r^3} = \frac{3}{r} \quad (3)$$

If in our example we assume the radius of the small sphere to be 10 mm. and that of the large sphere to be 15 mm., we have as the relation between the surface area and the volume,

$$\text{for } r = 10, \frac{\text{surface area}}{\text{volume}} = \frac{0.3}{1}$$

$$\text{and for } r = 15, \frac{\text{surface area}}{\text{volume}} = \frac{0.2}{1},$$

or the rate would be 50 per cent more rapid for the small sphere than for the large, if the rate of loss of water is governed by the surface area per unit volume. The small orange fruits would have more surface area per unit of volume than the large ones, and would consequently have a greater opportunity to lose their water.

Considering table I in this way, for the small oranges $\frac{3}{r} = \frac{3.53}{1}$, and for the larger oranges $\frac{3}{r} = \frac{2.4}{1}$; hence the expected rate of water loss per unit volume from the small fruit would be 1.47 times that from the larger fruit. The table shows that for a 25-hour period the rate of water loss from the smaller fruits was 1.53 times that from the larger fruits. Discrepancies are doubtless due in part to the fact that orange fruits may deviate considerably from a sphere in shape. In table IV, in which fruits of approximately the same size were taken, we find practically the same rate of water loss, again confirming the assumption.

The surface area of orange fruit in relation to the volume is no doubt a determining factor during hot weather as to the degree of severity of the so-called "June drop." The insurance against this source of fruit loss during periods of high temperatures, when the leaves require most of the available water in the trees, lies chiefly in the direction of having the fruits set at as early a date as possible in the spring, so that when hot weather begins the fruits may be of advanced size. During periods of high temperature, when the evaporation from the leaves is very rapid, the drop of small fruits may be excessive, in spite of a favorable soil moisture supply. Experimentation as to the methods of producing an early set of fruit on orange trees may prove of considerable value.

Summary

1. The percentage loss of moisture from young oranges and grapefruit decreases rapidly as the fruits increase in size. This change doubtless is due largely to the decrease in proportionate surface area as the fruit increases in diameter.
2. Observations on tagged fruits confirm the common knowledge that young citrus fruits readily undergo abscission up to a rather definite stage of size development, but only rarely thereafter.
3. It is possible that evaporation rate is an important factor in determining susceptibility to abscission.
4. Stomatal regulation or the nature of the rind is not an important factor in determining the rate of water loss from detached fruits of different sizes.
5. The size reached by grapefruit before the time of extremely high temperatures may determine to a large extent the occurrence of fruit clusters. Detached citrus fruits of approximately the same size lose their moisture at approximately similar rates.
6. The moisture content is very similar in young orange fruits of different sizes.
7. Any climatic conditions, fertilization program, or cultural operations that may hasten the sizing of the young fruits, may give them a better opportunity to remain attached to the tree when high daily maximum temperatures are experienced.

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BRIEFER ARTICLES

THE AUXIMONE THEORY

The work of BOTTOMLEY and his pupils on the subject of plant growth-promoting substances, or "auximones," has recently been the subject of some criticism in this journal at the hands of WOLFE (10). As the only pupil concerned, and one concerned very largely with the work of BOTTOMLEY as well as with independent work, the writer cannot agree that the last word has been said on the subject, in spite of WOLFE's suggestion that the term "auximones" may well be dropped from the literature.

It is fairly evident that it is their interpretation of the experimental facts recorded in the various publications of BOTTOMLEY and the writer on the subject which is called into question, and not the accuracy of these facts. In justice to the late Professor BOTTOMLEY, however, it seems advisable that it should be made clear that it was only after his original experiments with *Lemna minor*, carried out in his own laboratory, had been repeated on a greatly extended scale in an independent laboratory open to the inspection of his botanical confrères, and the repetitions had, under these conditions, demonstrated convincingly the truth of his statements, that his first publication on work with *L. minor* appeared in 1917 (1). Since that time, the work which BOTTOMLEY and the writer have carried out, jointly and independently, has all supported the main conclusions which were then reached.

BOTTOMLEY's interpretation of the term "auximone" is brought out in his statement in 1915 (2), that "the plant food accessories resemble more closely the growth-stimulating food factors of HOPKINS than the "vitamines" of FUNK, and the term "auximone" (Gr. *αὐξημός*, promoting growth) is suggested for them, being descriptive of their action rather than of their nature or composition." This interpretation of their rôle, as being necessary for optimum growth and development rather than for the actual maintenance of life, which was BOTTOMLEY's real intention, and which falls into line with CLARK and ROLLER's (5) conception of the accelerating action of the accessory substance for yeast, becomes apparent on careful perusal of his later work, and is supported by various statements of the writer (7).

At least one other worker has observed an increase in growth as a result of additions to mineral nutrient solutions of materials similar to those which BOTTOMLEY and the writer have used as a source of auximones. Also, since a "catalytic" effect brought about by additions of peat extract and autolyzed yeast in the hands of SAEGER (9) is admitted as a possibility by WOLFE, it is difficult to see why the same effect, obtained by BOTTOMLEY and the writer, and called by them a "growth-promoting" or "auximonic" effect, should be subject to criticism. The unknown nature of these substances, though, as WOLFE states, it may aggravate the difficulties, is surely no condemnation, in view of the mystery in which the nature of the "vitamines," so widely accepted as essential factors in animal metabolism, has for so long been shrouded; while the fact that certain organic substances of known constitution have, in WOLFE's hands, failed to result in any increase in growth, is no proof that all organic substances, of known or unknown composition, will do so. The fact, reported by WOLFE, that certain organic substances, in concentrations of 50-200 p.p.m., actually depressed the rate of growth of *Lemna* plants as compared with that in mineral nutrients only, is significant as indicating the potency of small quantities of organic substances, for good or ill, in plant metabolism, although WOLFE states that the small quantities added can hardly have exercised any specific toxicity. Since precisely similar quantities of organic materials of unknown nature have been responsible for a very marked stimulation, in the experiments of BOTTOMLEY and the writer, the acceptance of a toxic effect of such materials and in such quantities as those specified presents no difficulty to the writer.

There are one or two points which need to be stressed with regard to the work of SAEGER (9) and MENDIOLA (6) which has been chiefly quoted by WOLFE (10). One of the most important precautions taken by BOTTOMLEY and the writer in the course of their work has been the frequent changing (not less than twice weekly) of the culture solutions used, not so much to maintain a balance of materials as to eliminate as far as possible the contamination by bacteria and algae which they found to result inevitably from a less frequent changing of solutions. Such contamination affects materially the results of the experiments, for both BOTTOMLEY (3) and the writer (8) have shown that very small quantities of the products of certain bacteria have a marked effect in stimulating growth, while certain hitherto unpublished observations on the part of the writer suggest that contamination with blue-green algae has a somewhat similar effect. In any case, it has been a matter of frequent observation that control cultures in mineral nutrients only, when left so neglected at the conclusion

of an experiment that they became visibly contaminated with bacteria and algae, improved markedly in health. Another point that BOTTOMLEY (2) has stressed is the fact that it was frequently not until after the lapse of at least 21 days that any appreciable difference in the size of the plants in the different cultures became manifest. The writer's experience goes to show that experiments lasting over a period much longer than 21 days are necessary for any sound conclusions to be drawn.

The variability in the rate of reproduction of *Lemna* rendered it important that the number of cultures for any single nutrient solution should be made sufficiently large to enable an average rate of reproduction to be obtained. In the work of the authors in question, details with regard to these points are not always given, but in some cases such details as are given show clearly that the precautions mentioned were not always observed. For instance, experiments of 23 days' duration are quoted, while SAEGER claims that he was able to grow *Spirodela polyrhiza* for 26 months in Knop/10 solution changed "at intervals usually of one or two weeks," periods which the writer has found, during more than ten years' experience with such work, to be long enough to induce contamination sufficient materially to affect the result, for it must be emphasized that relatively minute amounts of organic material have been found to bring about a marked stimulation.

The results of the experiments of SAEGER on the effect of the additions of extracts of peat and of yeast to mineral nutrient solutions support materially the conclusions of BOTTOMLEY and the writer. His comparison of the effect of an inorganic medium as compared with pond water is also in accordance with the results of BOTTOMLEY (1), although apparently he would not agree as to this interpretation of them. SAEGER's implied suggestion, that the beneficial effect of the yeast lay in the possibility that it contained some ash constituent which supplied a deficiency in the culture solution, is negatived by the report of BOTTOMLEY on the absence of any stimulating effect due to the ash constituents of *Azotobacter* and of nucleic acid, while these materials themselves had a marked effect as growth stimulators. Similar unpublished experiments were made with the ash of yeast, with similar negative results.

Attention has been drawn to these points in view of the fact that this work, and that of CLARK and ROLLER, has been used very largely by WOLFE to support his contention that BOTTOMLEY's theory is completely refuted, and that the advantage of the organic matter in BOTTOMLEY's experiments lay in its restoration of the physiological balance of a solution which, it is suggested, was about ten times too concentrated. In the work

quoted, the writer sees no justification for such a claim, which appears to be based largely on a misinterpretation of BOTTOMLEY's use of the term "auximone"; but if it is indeed a fact that quantities of organic matter ranging around about 100 p. p. m. (a fair average in BOTTOMLEY's experiments) may have such an effect in neutralizing the ill effects of such over-concentration of mineral nutrients, then BOTTOMLEY's work has been worth while in drawing attention to such a fact.—FLORENCE A. MOCKERIDGE, *University College of Swansea, Swansea, Wales.*

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STAINING OF YEAST CELLS

Methods of staining yeast cells have been collected to 1915 by EYRE.¹ Further improvements in technique have been made by DAVIS² on spore staining which are applicable to yeasts, and by WINGARD³ in his study of the nuclear phenomena of the yeasts.

With large elementary classes, the smear method, whereby a drop of the culture is evaporated on a cover slip and fixed in the flame, usually results in shrinkage and distortion of the cells, and the cell content is not readily discernible. A method which is rapid and practicable for use in large classes has been devised in the botanical laboratory of Rutgers University. This involves the same principle as that used by DAVIS, who imbedded the cells in a thin film of parlodion on a slide, but requires less time. By it there results but little shrinkage of the cell, and vacuoles and protoplasmic structures are easily defined.

The procedure is as follows. On a clean glass slip place a drop of dilute albumin fixative (Mayer's albumin, diluted 1:10 with distilled water), stir in a small drop of the yeast culture and allow to dry over gentle heat (as on a warm radiator). The material should then be stained (acid fuchsin, acting for 50 seconds gives good results), the excess washed off, then dried as before, and mounted in balsam.—ARTHUR P. KELLEY and M. B. SHOEMAKER, *Rutgers University, New Brunswick, N.J.*

¹ EYRE, J. W. H., *The elements of bacteriological technique*. Pp. x+518., figs. 218. Philadelphia: W. B. Saunders Co. 1915.

² DAVIS, W. H., *Staining germinating spores*. *Phytopath.* 12:492-494. 1922.

³ WINGARD, S. A., *Studies of the pathogenicity, morphology and cytology of Nematospora Phaesoli*. *Torr. Bull.* 52:249-290. 1925.

CURRENT LITERATURE

BOOK REVIEWS

General botany textbook

A volume¹ has recently appeared which is an amazing compendium of botanical information. To review it critically in a few paragraphs is quite impossible. One can only give some indication of its content and method of treatment.

Part I, the first 26 pages, outlines the problems of the science of botany. Part II, pages 29-297, discusses the vegetative functions of plants, and is excellently done. Part III, pages 301-554, takes up reproductive processes and life histories. Part IV reviews the great groups of seed-bearing plants, citing particularly those species of economic importance. Part V, pages 935-1012, is devoted to genetics and evolution.

The purposes of the authors, as stated in the preface, are to teach students how to think and to impart culture. "A subject has cultural value in proportion to the number of human contacts it gives the pupil, the extent to which it broadens his views and extends his interests and sympathies." The plants directly of service to man are the ones chiefly studied in the text, and attention is constantly called to the ways in which man is dependent upon them, and how profoundly they have affected the course of civilization.

Current errors in botanical instruction are noted and the misconceptions corrected, a feature that makes the book particularly valuable to the teacher whose instruction in botany was received several years ago. Thus osmotic pressure is not to be considered merely as a physical effect, produced by the bombardment of the membrane by the molecules of the liquid, but that the plant membrane acts as a solvent, and substances may pass by osmosis only when they are soluble in the membrane. Again, roots penetrate soils to a depth of three feet or more, six or eight feet when necessary to obtain water and food materials, so the practice of subsoiling, deep tillage, even soil dynamiting is unnecessary, as shown by the investigations of CHILCOTT and COLE (p. 129).

Citations of investigations are abundant, as in the case just given, and the footnotes give details of the place and date of publication. There is also a definite effort to introduce many historical references as a means of achieving the cultural aim. "The causal relationship between gravity and the direction of roots and shoots was first established by the English botanist THOMAS ANDREW KNIGHT," who devised the familiar experiment of directing growth by centrif-

¹ GAGER, C. S., General botany, with special reference to its economic aspects, and three chapters on genetics by ORLAND E. WHITE. pp. xvi+1056. P. Blakiston's Son & Co. 1926.

ugal force. A footnote gives a brief but illuminating sketch of the man. Such historical matter is found not only in footnotes but in the body of the text.

Throughout the book economic botany receives prime consideration, not because the book is intended for those students who are to become farmers or horticulturists, or who will follow some other application of economic botany professionally, but because the author conceives that the beginning student is interested best by dealing with those plants and those topics that relate botany most closely to men's needs. The author is not afraid to overstep the bounds of botany in following practical applications into related fields. Thus, under the topic Pollen and hay fever (p. 40), sensitivity to animal proteins is discussed. A patient is cited who was put to bed with hay fever in August by ragweed, but kept there until April by the feather pillows.

The book presupposes a laboratory course as an accompaniment of the text, from which "the student will get at first-hand many facts not given in the text." Certainly with such a combination a beginning student who masters both should have a good grip on the fundamentals of botany. It would make a formidable course, and the very bulk of the book may act as a deterrent to its wide adoption. It is an excellent text, however, for ambitious students.—E. R. DOWNING.

Plant life in the Alps

It is fitting that 50 years of botanical investigation, teaching, and writing, should be crowned with the production of a masterpiece. CARL SCHRÖTER began his career as a botanist almost exactly 50 years ago, and on December 19, 1925, he retired from his position of Professor of Botany in the Technischen Hochschule at Zurich, on his 70th birthday. Almost simultaneously there appeared the enlarged second edition of his great work on alpine plants.² It is essentially a new book, as the additions and revisions have added nearly 500 pages to the 800 which formed the edition of 1908.

In such a large and comprehensive work it is difficult to give any adequate idea of the scope and variety of its contents. Many topics are discussed and every one in an exhaustive manner. The contributions of others as well as the wide experience of the author are called upon to make these discussions broad and accurate. As an example, there may be cited the contents of a single table in which the data of not less than thirty investigators on the altitudinal limits of various types of vegetation are displayed in parallel columns. This is but a small fraction of the discussion of the vexed question of the causes and limits of alpine timberline.

Beginning with a general discussion of the distribution of the various types of vegetation in the Alps, climatic, biotic, and soil factors are considered. These different factors are closely analyzed, and their effect on various plant associations indicated. There follows a detailed discussion of the various elements comprising the alpine flora. Here will be found a series of monographs on the habits,

² SCHRÖTER, CARL, *Das Pflanzenleben der Alpen. Eine Schilderung der Hochgebirgsflora.* 2d ed. pp. vii+1288. pls. 6. figs. 316. Zurich: Albert Raustein. 1926.

structure, ecology, variation, and distribution of every species of importance in the entire flora. This includes not only woody and herbaceous flowering plants, but also ferns, mosses, algae, and fungi.

Special attention is given to the variations in form and structure occurring in the forest, in the subalpine and alpine scrub, in the meadows, in the alpine mats, in the fell fields, and in other characteristic communities. Structural responses to temperature, wind, moisture, light, and length of growing season are carefully considered. Each topic is so thoroughly treated that it is hard to point out special features, although the exhaustive consideration of cushion and polster forms is probably equaled in no other work.

A final section of the volume, comprising about 100 pages, is contributed by HEINRICH and MARIE BROCKMANN-JEROSCH, and covers the origin, affinities, and distribution of the Swiss alpine flora. It includes a consideration of the influence of the glacial period, and the distribution and migrations of the flora before, during, and since this glacial epoch.

The bibliography is extensive, well arranged, and brought down to include the articles in Schröter's *Festschrift*,³ many of which relate to problems of alpine vegetation.

The entire mass of material covering the thousand closely printed pages is so well organized and so thoroughly indexed as to make reference easy. It is indeed a compendium of all matters relating to alpine vegetation, and without a rival in this particular field. It is safe to predict that it will long remain the authority on the problems connected with the vegetation of higher mountains the world over.—G. D. FULLER.

Chemistry of wood

An important pioneering piece of work has been done by HAWLEY and WISE,⁴ in bringing together the data on the chemistry of wood. Their book represents the first attempt to summarize in one volume the literature on this subject.

The book is divided into five parts. Part I is the introduction. In part II is considered the chemical components of wood, including cellulose, polysaccharides, lignin, and certain extraneous components such as resins and essential oils, fixed oils, fats, and other compounds. In regard to the middle lamella of woody tissue, reference is made to the work of RITTER, who concluded that the compounds making up the middle lamella should be classed with lignin rather than with the pectins, and that the claim of botanists that the middle lamella is made up mainly of pectin or calcium pectate is not sufficiently supported by experiments. The literature on lignin is well summarized. Various formulas for lignin, which have been tentatively proposed, are given. It is pointed out that

³ BROCKMANN-JEROSCH, H. (editor), *Festschrift CARL SCHRÖTER*. Veröffent. Geobot. Institute Rübel Zürich 3:1-36. 1925.

⁴ HAWLEY, L. F., and WISE, L. E., *The chemistry of wood*. pp. 334. New York: Chemical Catalog Co. 1926.

none of these formulas can be considered as established, and that the term lignin cannot be applied to any definite compound present naturally in the cell wall. The various color reactions which have been used as tests for lignin are referred to, and it is shown that in the main they are probably due to an aldehyde which makes up a very small part of the lignin. A negative color test is not necessarily an indication that lignin is absent, that is, that delignification has been complete. Also, the color tests are not very specific, for some of them may be positive with natural substances, for example, oil of cloves and oil of sassafras; so the tests need to be used with caution.

Part III considers methods of analyzing wood for the various components, including cellulose, the pentosans, hexosans, and lignin, with some of the practical applications of such analyses. Part IV takes up the decomposition of wood, considering its combustion, with the mechanism of the process, the destructive distillation, the hydrolysis and delignification, and its decomposition with concentrated alkali. In regard to the hydrolysis of cellulose, cotton cellulose is used first as an example, and data are referred to, showing that even by dilute acid considerable cellulose is hydrolyzed. Part V considers wood as an industrial material, taking up certain physical properties, such as strength, specific heat, conductivity, absorption of water and other liquids, and including a consideration of decay.

The book is much more than a mere summary of the literature. The literature is critically evaluated. The present status of our information on the subject is shown, and the gaps in our present knowledge pointed out; thus further research along this line should be stimulated. The volume should be a valuable reference book for the general botanist, and an invaluable tool for the forester and technical worker in the commercial processes using wood as a raw material.—S. V. EATON.

Principles of plant growth

An elementary text on botany^s has been published by ROBBINS, whose title suggests its major purpose. The "principles of plant growth" are presented in non-technical language, so that they may be understood by all who are interested in the growing of plants. This makes the book useful, not only as an elementary text in secondary schools, but also to all engaged in the growing of plants, as farmers, nurserymen, orchardists, etc. No attempt is made to give the structures of plants in minute detail, but the work carried on by the different parts is presented very simply and clearly. As an elementary presentation of the main activities of plants in connection with growth this text is certainly a success.

The recommended sequence of presentation is indicated by the organization of the text. It begins with an account of the living plant body and its work, and the style is very popular, presenting the general facts in such a way as to be

^s ROBBINS, W. W., *Principles of plant growth, an elementary botany*. 8vo. pp. vi+299. figs. 136. New York: John Wiley & Sons. 1927. \$2.25.

appreciated by those with no training in botany, or even in science. Following this general account of the plant body, there are chapters on absorption by the roots, food building, substances taken into the green plant and the uses made of them, movement of sap in plants, transpiration, and respiration.

After this outline of the ordinary growth activities, there are chapters dealing with the roots, stems, and pruning, flower (including pollination and fertilization), seed (including germination and seed testing), propagation, and weeds. Other chapters deal with the relation of water, temperature, and light to plant life.

All of the preceding chapters naturally deal with the activities and structures of flowering plants, as those with which plant growers are chiefly concerned. The book closes, however, with a few chapters presenting the other groups, from algae to gymnosperms, so that the reader is introduced briefly to the lower plants.

This text will certainly prove of service in extending the knowledge of botany into groups that are not reached by the ordinary texts.—J. M. C.

NOTES FOR STUDENTS

Pollen analysis and postglacial vegetation.—The various changes in the vegetation of the northern portion of the British Isles and of the Scandinavian Peninsula during postglacial times have been subjects of very considerable interest, both for geologists and botanists. As the results of extensive investigations of the peat deposits of Scotland, SAMUELSSON⁶ confirmed and supported GEIKIE's conclusions that at two separate periods there had been more extensive forests than at the present time, indicating two periods of milder climate separated by a period of greater cold. He also showed that this exactly parallels conditions in southern Sweden, as determined by BLVTT.

Somewhat later, VON POST⁷ turned his attention to the pollen grains present in the peat, and elaborated methods of pollen analysis. It is argued that the pollen present in the different layers of peat will afford a better picture of the general tree vegetation of the period than is afforded by the remains of wood and fruit, since the pollen is carried by the wind and evenly distributed by chance. By such methods, VON POST was able to give a more detailed picture of Swedish forests during the warm postglacial periods termed "sub-boreal" and "Atlantic" by BLVTT. In the latter period *Quercus*, *Tilia*, and *Ulmus* seem to have been abundant in Sweden. Further confirmatory details were added in a subsequent study,⁸ in which regional areas of such postglacial forests were distinguished

⁶ SAMUELSSON, G., Scottish peat mosses. Bull. Geol. Inst. Univ. Upsala 10:197-260. 1910.

⁷ VON POST, L., Skogsträdspollen i sydsvenska torvmosselagerföljder. Geol. Fören. Förhandl. 38:384-394. 1916.

⁸ VON POST, L., Ur de sydsvenska skogarnas regionala historia under postarktisk tid (Eng. summary). Geol. Fören Förhandl. 46:83-128. 1924.

and mapped. It was clearly demonstrated that the mixed oak forest (*Quercus*, *Tilia*, and *Ulmus*) reached its culmination in "Atlantic" time, decreased somewhat in the subsequent "sub-boreal" time, attained another maximum in "early sub-Atlantic" time, and has been steadily decreasing ever since.

The methods used in these studies have been described in detail by ERDTMAN,⁹ and more recently by STARK.¹⁰ The peat or silt has often to be boiled in 10 per cent KOH and the residue placed in glycerin for microscopic examination. At times centrifuging may be employed without vitiating the results. The data of the microscopic examination and from the counting of the pollen grains are expressed in pollen diagrams, showing the relative abundance of the pollen of particular species at different horizons of the peat; by pollen spectra showing the relative abundance of pollen of different trees in a particular area; and by pollen maps showing the relative frequencies of different pollens in various areas at any particular time. Directions are given for the construction of such diagrams, spectra, and maps. Among numerous investigations by the same workers, interest seems to center in a preliminary study by ERDTMAN,¹¹ showing the similarity of the pollen spectra obtained from the peat of southwestern Sweden, northern Scotland, and Ireland,¹² and in a more detailed report on the post glacial forests of northern Scotland. A brief examination of some peat deposits on the Isle of Man¹³ reveals a close resemblance in its pollen spectra to those of the early "Atlantic" of Sweden, which are estimated to have been deposited 6500-9500 years ago. Investigations in Denmark by JESSEN¹⁴ also show that its deposits are in accord with the BLYTT-SERNANDER hypothesis of climatic succession. An excellent table here serves to make the various correlations clearly evident. All these results tend to show that in early postglacial times such trees as *Quercus*, *Tilia*, *Ulmus*, and *Fagus* had a much more northern distribution in the British Isles than at present.

ERDTMAN¹⁵ has further shown by the use of similar methods that there was

⁹ ERDTMAN, O. G. E., Pollenanalytische Untersuchungen von Torfmooren und marin Sedimenten in Südwest-Schweden. *Arkiv. Botanik* 17:1-137. 1921.

¹⁰ STARK, P., Der gegenwärtige Stand der pollenanalytischen Forschung. *Zeitschr. Bot.* 17:89-125. 1925.

¹¹ ERDTMAN, O. G. E., Studies in micro-palaeontology I-IV. *Geol. Fören. Förhandl.* 46:676-681. 1924.

¹² ———, Studies in the micro-palaeontology of post glacial deposits in northern Scotland and the Scotch Isles, with special reference to the history of the woodlands. *Jour. Linn. Soc. Bot.* 46:449-504. 1924.

¹³ ———, Pollen statistics from Currogh and Ballaugh, Isle of Man. *Proc. Liverpool Geol. Soc.* 14:158-163. 1925.

¹⁴ JESSEN, K., Mose undersøgelser in det nordøstlige Sjælland (Eng. summary: Bog investigations in northeast Sjælland). *Danmarks Geol. Unders. Series 2. no. 34. 1-241.* 1920.

¹⁵ ERDTMAN, O. G. E., On the immigration of some British trees. *Jour. Bot.* 64: 71-74. 1926.

an early postglacial increase of *Corylus* pollen in the peat of middle and northern England, showing that it was one of the first forest elements to have reached these regions in its northern migration. It seems to have been followed by *Ulmus*, *Quercus*, *Alnus*, *Tilia*, and *Fagus* in the order named.

Confirmation of these results has come from other portions of Europe. Working in Bohemia, RUDOLPH and FIRBAS¹⁶ found indications of former higher altitudinal limits for forests in the Erzgebirge Mountains. More recently, the same workers¹⁷ investigated the reliability of the pollen analysis hypothesis by examining recent peat deposits in various forest regions. They concluded that, in spite of minor discrepancies, the pollen precipitate at various altitudes reflects with considerable accuracy the composition of adjacent forests. The examination of peat deposits taken at depths up to 240 cm. in the moors of the high Giant Mountains gave pollen diagrams indicating that such trees as *Pinus*, *Fagus*, and *Quercus* had formerly an altitudinal limit at least 500 m. above that at present existing. This is in agreement with other data indicating that in Bohemia also there was a warmer postglacial period. Similar indications have been obtained as the result of the examination of a fresh water lake silt deposit, termed "Gyttja," and composed almost entirely of organic material. It was taken from marshes representing this former fresh water lake near the southern foot of Erzgebirge Mountains. The author¹⁸ here discusses the possibility of dating these deposits with some accuracy by means of fragments of pottery associated with the pollen in the lacustrine deposits.

That this is possible has been shown by VON POST, using pollen spectra from Scandinavia for dating and synchronizing. A further application comes from the work of WOODHEAD,¹⁹ and of WOODHEAD and ERDTMAN²⁰ on the peat deposits of the southern Pennines, near Huddersfield, England. Here the pollen diagrams indicate that the lowest of the peat was formed during the warm Atlantic period. Arrowheads and similar remains in this peat are of Neolithic Age. According to DE GEER, the Atlantic period may have lasted for some 3000 years, or about 5200 to 2200 B.C.

These results from the peat deposits seem to warrant interesting data from the application of similar methods in America.—G. D. FULLER.

¹⁶ RUDOLPH, K., and FIRBAS, F., Palaeofloristische und stratigraphische Untersuchungen böhmischer Moore: Die Hochmoore des Erzgebirges. Beih. Bot. Centralbl. 41:1-162. 1924.

¹⁷ ——, Pollenanalytische Untersuchungen subalpiner Moore des Riesengebirges. Ber. Deutsch. Bot. Gesells. 44:227-238. 1926.

¹⁸ RUDOLPH, K., Pollenanalytische Untersuchungen in thermopilem Florengebiet Bohmens: Der "Kommerner See" bei Brux. Ber. Deutsch. Bot. Gesells. 44:239-248. 1926.

¹⁹ WOODHEAD, T. W., The age and composition of the Pennine Peat. Jour. Bot. 62: 301-304. 1924.

²⁰ WOODHEAD, T. W., and ERDTMAN, O. G. E., Remains in the peat of the southern Pennines. Naturalist 245-253. 1926.

Taxonomic notes.—SMALL,²¹ in connection with his investigations of the flora of Florida, has discovered a new species of *Lupinus* and of *Polygala*. It is interesting to note that of the 27 species of *Polygala* growing naturally in Florida, about 12 are endemic.

In continuation of his studies of West Indian plants, BRITTON²² has published descriptions of 21 new species from Cuba, in 18 genera; 10 new species from Trinidad, in 9 genera; and a new tree from Porto Rico.

In presenting what are called the *arvensis*-series of British species of *Viola*, DRABBLE²³ recognizes 10 species, 2 of which are described as new.

In continuation of his description of the GOSSWEILER collection of plants from Angola and Portuguese Congo, GOOD²⁴ has described a new genus (*Ap-punettia*) of Rubiaceae, and also 9 new species.

SMALL²⁵ has described a new species of *Peperomia* (*P. floridana*) from Florida. He states that this "large tropical genus has crossed the Gulf Stream sparingly and settled in Florida in 5 species."

COCKERELL²⁶ has described a new genus (*Phenacocladus*) of algae from the Green River Eocene Rocks of the Roan Mountains, Colorado. It resembles certain marine forms, but occurred in a lake. COCKERELL concludes that the waters of the lake were saline, and that "the Green River aquatic fauna and flora were remote descendants of a group of marine organisms that had become isolated."

GARDNER²⁷ has begun a series of publications describing new species of Rhodophyceae from the Pacific coast of North America. The first number contains descriptions and plate illustrations of 10 new species in the following genera: *Helminthora* (2), *Helminthocladia* (1), *Myriogramma* (1), *Erythroglossum* (2), *Membranoptera* (4).

REHDER²⁸ has published the third part of his presentation of the ligneous plants of northern China, including Leguminosae to Sapindaceae. It presents

²¹ SMALL, J. K., Two new species from Florida. *Torreya* 26: 91-93. 1926.

²² BRITTON, N. L., Studies of West Indian plants. XIII. *Bull. Torr. Bot. Club* 53: 457-471. 1926.

²³ DRABBLE, ERIC, Notes on the British pansies; the *arvensis*-series. *Jour. Bot.* 64: 263-271. 1926.

²⁴ GOOD, R., Mr. JOHN GOSSWEILER'S plants from Angola and Portuguese Congo. *Jour. Bot. Suppl.* 64: 25-32. 1926.

²⁵ SMALL, J. K., An additional species of *Peperomia* from Florida. *Torreya* 26: 109. 1926.

²⁶ COCKERELL, T. D. A., An alga from the Eocene of Colorado. *Torreya* 26: 114. 1926.

²⁷ GARDNER, N. L., New Rhodophyceae from the Pacific coast of North America. I. *Univ. Calif. Publ. Bot.* 13: 205-226. *pls. 15-21.* 1926.

²⁸ REHDER, ALFRED, Enumeration of the ligneous plants of northern China. III. *Jour. Arnold Arboretum* 7: 151-227. 1926.

full details of bibliography and stations, and also the distribution of the species beyond northern China. Much the largest family is the Leguminosae, including 80 species, 4 of which are new, the only new species (*Caragana* and *Wistaria*) described in the paper. The 16 families presented include 52 genera and 185 species, much the largest genera being *Erythronium* (29 species), *Caragana* (22 species), *Acer* (21 species), and *Lespedeza* (13 species). The contribution is a very full and up-to-date record of these families for that region.

JOHNSON²⁹ has concluded that *Saxifraga Nuttallii* Small should be made the basis of a new genus, to which he gives the name *Cascadia*, for the Cascade Mountains. Its habitat is the Willamette Valley in Oregon and the region bordering on the lower Columbia River.

REEDER³⁰ has described a new genus (*Monimopetalum*) of Celastraceae from China, which is closely related to *Erythronium*.

In his sixth paper describing new plants from Central America, STANDLEY³¹ describes 13 new species in 10 genera. The most interesting one is *Parmentiera Valerii*, which is "a large tree belonging to a group hitherto represented in Central America by only two known species, both of which are trees much inferior in size to this one." Only one tree was seen, and "this was too large to be climbed, and it was only by throwing sticks at the high crown that imperfect specimens of the leaves could be obtained." This is a good illustration of some of the difficulties of collecting.

COOK and HUBBARD³² have described 5 new species of *Gossypium* from the west coast of South America, in Colombia and Ecuador. They state that the native cottons of that region are not closely related to Mexican species, and also show characters not previously recognized in the genus.—J. M. C.

Trees and shrubs of Mexico.—STANDLEY³³ has published the fifth and final installment of the *Trees and shrubs of Mexico*. This volume brings together all the published species of woody Mexican plants, based on the series of Mexican plants in the National Herbarium. As stated in the preface, "the flora of Mexico, especially the arborescent flora, includes many species of great economic value," and for this reason the presentation includes not merely the taxonomic details, but much information concerning commercial and local uses of the plants. Since it is an assemblage and organization of material rather than a

²⁹ JOHNSON, A. M., The status of *Saxifraga Nuttallii*. Amer. Jour. Bot. 14:38-43. figs. 2. 1927.

³⁰ REEDER, ALFRED, *Monimopetalum*, a new genus of Celastraceae. Jour. Arnold Arboretum 7:233. 1926.

³¹ STANDLEY, P. C., New plants from Central America. VI. Jour. Wash. Acad. Sci. 17:7-16. 1927.

³² COOK, O. F., and HUBBARD, J. W., New species of cotton from Colombia and Ecuador. Jour. Wash. Acad. Sci. 16:545-552. 1926.

³³ STANDLEY, P. C., Trees and shrubs of Mexico (Bignoniaceae—Asteraceae). Contrib. U.S. Nat. Herb. 23:1313-1721. 1926.

monograph, very few new species are described, only twenty in this last part. Several collaborators have contributed certain groups in which they have specialized.

In the present part 8 families are presented, representing 205 genera and 1327 species of woody Mexican plants. Far the largest family is the Asteraceae (Compositae), with 112 genera and 972 species. The Rubiaceae are second in abundance, being represented by 46 genera and 196 species. There are also 40 pages of "additions and corrections," bringing the information up to date. The extent of these additions and corrections indicates the great activity of our collectors and taxonomists. The volume is a most valuable compendium of information in reference to the Mexican flora.—J. M. C.

Vegetation illustrated.—More than 20 years ago there appeared the first issues of the *Vegetationsbilder*, whose illustrations established a new standard of excellence in depicting the vegetation of various regions. The most recent issues, beginning the seventeenth volume, continue the same high quality of illustrations. The first fascicle³⁴ gives some of the typical vegetation of the Crimea, and includes woodlands in which are found *Juniperus excelsa*, *Pinus laricio*, and *P. Pithysa*, together with mountain plateaux, steppes of *Artemisia maritima*, and salt meadows. In another issue³⁵ the steppes and semidesert of parts of Russia are described and illustrated, while in a third³⁶ the coast vegetation of southern Dalmatia is depicted. This last shows *Beta maritima* growing wild on the Adriatic coast, and includes among the species illustrated *Capparis rupestris*, *Allium ampeloprasum*, *Ihula candida*, *Centaurea Fridrici*, *Alyssum leucadeum*, *Helichrysum italicum*, *Pistacia lentiscus*, and *Euphorbia dendroides*.—G. D. FULLER.

Forest trees of Hokkaido, Japan.—This fine series by MIYABE³⁷ and his colleagues is presenting the trees of northern Japan in most attractive form. The present fascicles continue the beautiful colored plates already commended,³⁸ depicting the various stages of the trees from seedling through foliage and flowers to fruit. The four fascicles now issued include *Fagus Sieboldi*, *Castanea crenata*, *Quercus dentata*, *Q. magnolica*, *Q. crispula*, *Q. glandulifera*, *Ulmus japonica*, *U. laciniata*, *Celtis Bungeana* var. *jessoensis*, *Morus bombycis*, and *Cercidiphyllum japonicum*.—G. D. FULLER.

³⁴ WULFF, EUGEN, *Vegetationsbilder aus der Krim. Vegetationsbilder*, Karsten & Schenck 17:Heft 1. pls. 1-6. 1926.

³⁵ KELLER, B., *Die Grassteppen im Gouvernement Woronesh (Russland). Vegetationsbilder*, Karsten & Schenck 17:Heft 2. pls. 7-12. 1926.

³⁶ GINZBERGER, A., *Küstenvegetation der Süddalmatinischen Eilande. Vegetationsbilder*, Karsten & Schenck 17:Hefte 3, 4. pls. 13-24. 1926.

³⁷ MIYABE, K., and KUDO, Y., *Icones of the essential forest trees of Hokkaido*. Publ. by Hokkaido Government, Sapporo. 2:Fasc. 11. 1-6. pls. 32-34. 1925; Fasc. 12. 7-14. pls. 35-37. 1925; Fasc. 13. 15-22. pls. 28-40. 1926; Fasc. 14. 23-26. pls. 41-43. 1926.

³⁸ BOT. GAZ. 72:55. 1921; 75:431. 1923.

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BIOCHEMISTRY OF PLANT DISEASES

IX. PECTIC ENZYMES^a

F. R. DAVISON^b AND J. J. WILLAMAN

(WITH FIVE FIGURES)

I. Introduction

Pectin plays an important rôle in the life processes of plants, and for that reason the solution of some of the problems related to the pectic enzymes should throw some light on the physiological significance of pectin in plant life. The importance of these substances is shown by the fact that they are found almost as widely distributed as cellulose. Pectic compounds are essential constituents of cell walls of all the higher plants, being the main constituent of the middle lamella, and as such a cementing material between cells. It is here that the pectic substances have aroused the interest of the plant pathologist, because of the ability of certain parasites to penetrate tissues by virtue of their pectic enzymes.

The number and nature of pectic compounds are not known. Furthermore, the number of enzymes that act upon these pectins is disputed. Three of them have been described, but only two are admitted by some investigators. Our principal interest in them is from the standpoint of parasitism in plants, since the possession of one of these enzymes by a fungus may enable it to penetrate a host tissue,

^a Published with the approval of the Director, as Paper no. 647, Journal Series, Minnesota Agricultural Experiment Station.

^b The material in this paper was presented to the graduate school of the University of Minnesota by F. R. DAVISON, as his thesis for the degree of Doctor of Philosophy.

and the possession of another may gain for the fungus some nutrient from the pectins.

Because of the facts that pectins are so widely distributed, that they have lately been receiving considerable attention at the hands of investigators, that a knowledge of their enzymes is in a very unsatisfactory state, and that a better knowledge of them would aid in some of the problems of parasitism, it was thought desirable to initiate a study of them. The work reported here deals largely with their identity, and with the means of measuring their activity.

II. Definitions

At the present time there are generally recognized three enzymes acting upon the pectic substances. Considerable confusion exists in the literature concerning the naming of these enzymes. The names given by their discoverers are pectosinase, pectase, and pectinase. Although in general it is advisable to use names given by discoverers, in the case of the first enzyme mentioned it is believed preferable to make the terminology conform to that recommended by the committee on pectin nomenclature of the Division of Agriculture and Food Chemistry of the American Chemical Society. This also conforms more closely to the standard etymology of enzymes of using the suffix -ase with the root form of the substrate. Since the diction of enzyme literature can be no clearer than that of the substrates of the enzymes involved, it is believed to be in the interest of clarity to present the committee's definitions of both the pectic substances and their enzymes. These definitions are as follows:³

PROPOSED NOMENCLATURE (REVISED MARCH 8, 1926)

PECTIC SUBSTANCES.—A group designation for those complex carbohydrate derivatives which occur in plants, or are prepared from plants and which are characterized by the presence of galacturonic acid units. In the naturally occurring pectic substances, the galacturonic acid units apparently exist in an acid reacting complex associated with arabinose and galactose units. This acid complex may

³ As a member of the committee, the junior author had access to the definitions as submitted. Until adopted by the committee on nomenclature of the society they are to be considered as tentative.

occur as a free acid or as a metallic salt, but usually occurs in ester combination with methoxyl groups.

PROTOPECTIN.—The term applied to the water insoluble, unhydrolyzed, methoxylated parent pectic substances which occur in the cell walls of plants, possibly in combination with cellulose or other plant constituents, and which on restricted hydrolysis yield pectins.

PECTIN.—The term applied to the water soluble, methoxylated pectic substances occurring in plant tissues, or to the methoxylated pectic substances obtained by restricted hydrolysis of the protopectin, so regulated as to produce maximum solution of pectic substance with a minimum cleavage of methoxyl. The product may be a mixture of substances of varying methoxyl content; the term pectin or pectins is accordingly a group designation for all intermediate pectic substances between protopectin and pectic acid. It is proper, however, to refer to an individual of the group as a pectin.

PECTIC ACID.—The term applied to the water insoluble pectic substances obtained by the hydrolysis of pectin with the complete elimination of the methoxyl groups. Pectic acid may be variable in composition, according to the type of hydrolysis employed; accordingly there may be a number of pectic acids, all of which are methoxyl free.

PROTOPECTINASE.—The term applied to the enzyme which hydrolyzes or dissolves protopectin, with the resultant separation of the plant cells from each other, usually spoken of as maceration. Presumably the product of this hydrolysis is pectin. The term protopectinase supersedes the older term pectosinase with which it is synonymous.

PECTASE.—The term applied to the enzyme which converts pectin into pectic acid, the latter becoming a gel, especially in the presence of calcium (or barium or strontium) salts.

PECTINASE.—The term applied to the enzyme which hydrolyzes pectin and pectic acid into their simplest soluble cleavage products, which are probably arabinose, galactose, and galacturonic acid.

The writers feel that they have proved beyond reasonable doubt the existence of the three enzymes here defined; they will therefore use this terminology throughout the present paper. Since rather

complete reviews of the literature of the pectic enzymes have recently been published (1, 39), this literature will be referred to only incidentally during the presentation of the following data.

III. Protopectinase

OCCURRENCE AND PREPARATION

A macerating action on plant tissue has been reported for many species of bacteria and fungi (2, 3, 4, 24, 26, 29, 31, 37, 42). Recently protopectinase production has been the subject of extensive researches by HARTER and WEIMER (16-21), although they call it pectinase. They found that this enzyme is produced by several species of *Rhizopus*, that some of it is retained in the mycelium, and that a portion is excreted into the substratum. They also found it in the spores of *R. nigricans* and *R. tritici*. MISS PATON found this enzyme (called by her pectinase) in the eighteen species of pollen that she studied, and a histological examination showed that pollen tubes make their way between the adjacent cells rather than through them. SPAULDING (34) observed that in the last stages of wood decay produced by *Lenzites saeparia* Fr. the middle lamella has disappeared. ZELLER (43) found that this same fungus produces a substance dissolving the middle lamella of carrots and potato disks, coherence of the tissue being lost after 42 hours in an extract of the fungus powder.

A number of materials prepared by one or more of these methods were tested for protopectinase. The results are given in table I, using in this case a relative index of activity instead of definite time units for maceration. It will be noticed that this enzyme was found only in materials of plant origin. Its absence from the corn pollen should not be taken as conclusive, since this pollen was 7 years old at the time of testing.

This enzyme was prepared in three different forms: (1) Powdered mycelium produced by growing the organisms *Rhizopus tritici* and *Bacillus carotovorus* upon carrot decoction for 24-48 hours, then harvesting the mycelial mat, drying and grinding; this powder was extracted with water for use. (2) The liquid medium upon which the *Rhizopus* had grown. (3) The powder formed by precipitating the

enzyme from the medium with alcohol, filtering, drying, and grinding.

Large quantities of mycelial mat were prepared by inoculating sterile 250 cc. Erlenmeyer flasks containing 25 cc. of carrot decoction with spores from the young growth of the fungus to be used. At the end of the incubation period the fungus mat was harvested and washed in running tap water for 5 minutes, the water squeezed out, and then treated with acetone and ether, according to Dox's (13)

TABLE I
OCCURRENCE OF PECTIC ENZYMES IN MATERIALS TESTED

SOURCE	PROTOPECTINASE	PECTASE	PECTINASE
Rhizopus tritici.....	+++*	---	++
Aspergillus niger.....	---	---	+
B. carotovorus.....	+++	---	++
Sclerotinia cinerea.....	---	+++	+++
Clover leaves.....	---	+++	---
Pollen (corn).....	---	+++	---
Barley malt.....	---	---	++
Barley malt (fresh).....	---	+ (?)	++
Diastase of malt.....	---	---	---
Diamalt.....	---	---	---
Diastophore.....	---	---	---
Diazyme.....	---	---	---
Takadiastase.....	+	---	+
Emulsin.....	+	---	+
Ptyalin.....	---	---	---
Amylopsin.....	---	---	---
Steapsin.....	---	---	---

* +++ Very abundant; - absent.

modification of ALBERT and BUCHNER'S method. The method is as follows. The mycelia after being washed are immersed for 10 minutes in a large excess of acetone and constantly stirred, the hyphae being torn apart by forceps at the same time. The material is then squeezed dry and immersed in a fresh supply of acetone for 2 minutes with constant stirring. It is again squeezed dry and stirred for 3 minutes in ether, after which it is dried by a fan, then over sulphuric acid in a vacuum.

QUANTITATIVE DETERMINATION

The detection of this enzyme is based upon the fact that by dissolving the pectic lamella between the cells of certain plant tissues

the cells separate, and a maceration effect is produced. The methods used heretofore are qualitative rather than quantitative. BROWN (8, 9) and HARTER and WEIMER (17) used the time required for complete loss of coherence of the tissue submerged in the enzyme extract, judging the end point with their fingers. BROWN used disks of potato tubers and turnip roots 0.5 mm. thick, and HARTER and WEIMER used disks of sweet potatoes 1 mm. thick. The writers found it very difficult to get an accurate indication of the maceration by examining the disks, so it was finally decided to use the following technique for estimating maceration. A strip of potato tissue 0.5 mm. thick, 5 mm. wide, and about 20 mm. long was cut by the aid of a microtome and a knife made by clamping two safety razor blades over lead slugs the proper distance apart. By keeping the instruments very sharp, and taking slices very close together, tissue of uniform thickness and texture could readily be obtained. A strip thus cut was suspended by folding over the ends and fastening a small paper clamp on each end. Considerable care must be exercised in cutting the potato strips, and also in fastening the clamp so that there is a direct pull downward. A 10 gm. weight or its equivalent was hooked on one end, and the tissue and weight suspended in a test tube containing 10 cc. of the extract to be tested, and the whole placed in a thermostat. The time when the tissue disintegrated and the weight fell was noted. This is believed to be more accurate and to give more absolute results than the other methods mentioned.

IDENTIFICATION

In the list of materials in table I, it may be noted that *Sclerotinia cinerea*, the fungus causing the brown rot of plums, did not show any protopectinase activity. On the basis of photomicrographs of rotted tissue, VALLEAU (36) concluded that this fungus follows the line of the middle lamella in penetrating a tissue; and from this evidence WILLAMAN (40) predicted that a protopectinase would be found in the fungus, and MUHLEMAN (28) reported positive evidence of its presence. Its absence in these tests induced the writers to make a more careful search for it.

The pure culture of *Sclerotinia cinerea* was obtained from Dr. LOUISE DOSDAL of the Department of Plant Pathology of the Uni-

versity of Minnesota. Soaked dried peaches were used as a medium for the production of a supply of spores. For the culture medium an extract was prepared by stewing dried prunes and pressing out the juice and diluting to a Brix of 12°. Thirty cc. of this juice was placed in several 250 cc. Erlenmeyer flasks and autoclaved at 15 pounds for 20 minutes, cooled, inoculated with spores, and placed in an incubator at 25° C. for 2 days. The mycelia were then harvested, washed, and dried by Dox's method, and then dried in a vacuum over sulphuric acid. A 1 to 2 per cent extract was tested for its macerating activity qualitatively, by its macerating effect on potato and apple disks. No macerating activity could be detected.

CONDITIONS FOR GROWTH

The conditions for growth and maceration were then carefully studied to determine if possible the presence of a macerating enzyme, and the conditions necessary for its maximum production.

The hydrogen-ion concentration of the prune juice was adjusted according to the method employed by KARRER and WEBB (25). The most luxuriant growth was found at a P_H ranging between 3.5 and 6.5.

The effect of the concentration of the prune juice on mycelial growth was then studied. Several flasks in duplicate were adjusted to a Brix ranging from 1°-22°. These flasks were sterilized carefully and inoculated with *Sclerotinia cinerea*. The best growth was found between 7° and 18°, with no definite optimum.

A number of materials were tested to see which supported the most luxuriant growth of spores in the shortest time. The results are listed in table II. Peaches, pears, and apricots were soaked over night, peeled, and placed in Petri dishes and sterilized. After cooling they were then inoculated with the organism. From the results in table II it is seen that the best material for the propagation of spores is prune juice.

It was thought that lowering the osmotic pressure of the medium might improve the growth of the fungus. Peaches were therefore soaked in successive quantities of distilled water for 3 days previous to sterilizing. This had little effect on the growth; if anything it hindered it slightly.

Using prune juice as a medium and adjusting its acidity and density for maximum growth of the fungus, a great number of species of *Sclerotinia* and the closely related *Monilia* were tested for protopectinase activity. Most of these were obtained from DR. E. E. HONEY of Cornell University, who very kindly sent the cultures and a list of their origins. The list is given in table III. In addition plum mummies were obtained during the winter from University Farm and the Fruit Breeding Farm at Excelsior, Minnesota. These mummies were treated with 50 per cent alcohol to remove air bubbles, and the outside layer sterilized 2 minutes in 1:1000 mercuric chloride, then washed with sterilized water and placed in a moist cham-

TABLE II
SPORE PRODUCTION OF SCLEROTINIA CINEREA ON VARIOUS MEDIA

MEDIUM	RELATIVE SPORULATION	MEDIUM	RELATIVE SPORULATION
Potato dextrose agar.....	+	Potatoes (mashed).....	+
Prune agar.....	+	Sweet potatoes (mashed).....	+
Peaches (soaked) first sample	+++	Carrot (mashed).....	+
Peaches (soaked) second sample..	+++	Carrot extract.....	+
Peaches (soaked) third sample	+++	Beer wort.....	+
Pure agar.....	-	Peach extract.....	++
Pears (soaked).....	+	Apricot extract.....	+++
Apricots.....	+	Prune extract.....	++++

ber. A good growth of spores of *S. cinerea* was formed after several days, and a pure culture was easily procured.

In all cases the mycelia were harvested as soon as they covered the surface of the medium, usually 2 days. Extracts were made as noted, and tested for protopectinase. In none of the fungi could any trace of the enzyme be detected.

Since mycelia 2 or 3 days old showed no protopectinase, the procedure adopted by BROWN (8) was tried. He made a very thick suspension of spores in a nutrient medium, and allowed them to germinate just 24 hours. A heavy spore suspension was obtained by inoculation of sterile soaked dried peaches. A strong suspension of these spores was made by adding sterile distilled water and rubbing the spores loose; they were then inoculated into several flasks of prune juice. Sterile conditions were adhered to as closely as possible, but little contamination could result in the time given to the growth.

After 24 hours the growths were harvested, treated by Dox's method, and then dried in a vacuum over sulphuric acid, ground, and a

TABLE III

LIST OF FUNGI SUPPLIED BY DR. E. E. HONEY AND TESTED FOR PRESENCE
OF PROTOPECTINASE

CULTURE NO.	
H 193	Sclerotinia cinerea f. pruni from Dr. Wormald, East Malling, England; isolated from an ascospore; Wormald's culture no. 3995
H 194	S. cinerea f. mali from Dr. Wormald; culture no. 3989
H 201	S. cinerea from Dr. Westerdijk (Centraalbureau Schimmelkulturen), Baarn, Holland; data coming with this culture were as follows: "Sclerotinia cinerea (Bon) Schr. isolated by Miss Berkhouf from Persica spec. at Baarn. This culture came in our collection in 1922"
H 202	S. cinerea from Dr. Westerdijk (Centraalbureau Schimmelkulturen), with following data: "S. cinerea (Bon) Schr. Isolated May, 1921, from Prunus cerasus at Baarn"
H 209	S. cinerea collected apothecia near Ithaca, N.Y.; made single spore isolations from the apothecia on peach mummies, April 24, 1924
H 220c	S. cinerea collected apothecia on plum mummies from tree of Prunus triloba (var. Abundance), University Orchard, Ithaca, N.Y., May 10, 1924; made single ascospore isolations
H 238b	S. cinerea (Monilia stage); single spore isolations made from conidia produced upon mummmied plums, collected by Dr. E. F. Guba near Webster, N.Y., May 15, 1924; isolation by E. E. Honey
H 234	Monilia cinerea from Dr. Wormald, England, isolated from conidium
H 266	S. cinerea? causing blossom blight of apricot, collected by Mr. B. A. Rudolph, at Nicholson orchard, Aromas, Cal., and isolated by E. E. Honey from spore dilution cultures of conidia; Early Golden variety of apricot
H 205	S. laxa from Dr. Westerdijk (Centraalbureau Schimmelkulturen), with following data: "Sclerotinia laxa (Ehrenb.). We received this culture from Dr. Faes, Station federale d'essais viticoles, Lausanne, Switzerland, in March, 1924"
H 251	S. cinerea from Mr. J. C. Neill, Biological Laboratory, Wellington, New Zealand, with following data: "Cultures of Monilia stage of S. cinerea Schröt. From stock cultures originally made from diseased tissue of Prunus persica fruit. Auchland 30/1/24 collected and isolated by G. H. Cunningham"
H 295	Monilia oregonensis from Dr. H. P. Barss, Corvallis, Ore.; type called Monilia oregonensis by us; isolated from sporodochia on peach fruit, in orchard near Corvallis, January 27, 1924
H 195	S. fructigena from Dr. Wormald, England, his culture no. 3979; isolated from apple
H 323	Monilia fructigena from Dr. Wormald, isolated from plum by means of single conidium
H 233	S. fructigena from Dr. Wormald, isolated from pear by means of single conidium
H 322e	S. cinerea on peach from University Orchard, Ithaca, N.Y., May 3, 1925
H 234	Monilia cinerea from Dr. Wormald; cherry, April 23, 1925

strong water extract tested for protopectinase activity. Nearly half of the strains were tested in this way, but no activity could be detected.

It was thought possible that repeated growing of the various

strains of *Sclerotinia* on artificial media had caused a gradual degeneration of this enzyme; therefore an attempt was made to regenerate it. Several sound apples were cleaned and the surfaces well washed, and then placed in 1:1000 HgCl₂ for 2 minutes. After drying they were inoculated with spores through punctures in the skin, and placed under bell jars. Transfers were made successively through four apples. From each "generation" the fungus was isolated and tested for macerating ability. Both potato and apple tissue were used in the test, but no maceration could be detected.

BROWN (8, 9) found that the best reaction for maceration was the reaction of the extracts as obtained from the hyphae, and adjusting to any other P_H had no advantageous effect. He did not give the reaction that gave the maximum activity. Using extracts from *Rhizopus tritici*, the P_H was adjusted through a considerable range, as suggested by KARRER and WEBB (25), and checked by the potentiometric method. A blank determination was run in all cases. The data are given in fig. 1, in the top pair of curves. The greatest activity of the enzyme was at P_H 5, and gradually diminished on each side of this point. At the extreme alkaline and acid sides a maceration was evident with the enzyme absent. This action is probably due to direct maceration, since most of the extracts had a P_H of about 6.

It was thought that possibly the enzyme was secreted into the surrounding medium, and hence was not detectable in the mycelium. An apple well rotted by *Sclerotinia cinerea* was ground up, extracted with water, and tested for macerating principle, on both apple and potato tissue. No macerating action was detected up to 9 days. The medium upon which the spores had grown was also tested against potatoes, and again the results were negative.

As a last resort various strains of potatoes, of a wide range of mealiness, were used as test tissue for protopectinase activity, with the idea that the most mealy might have middle lamellae most easily hydrolyzed by the enzyme. The extract was made from dried ground mycelia in the usual manner from a young 24-hour growth of mycelia, and maceration tested on strips of potato from each variety. Also a cooking test was made to grade the mealiness of the potatoes. The results on all the potatoes were negative.

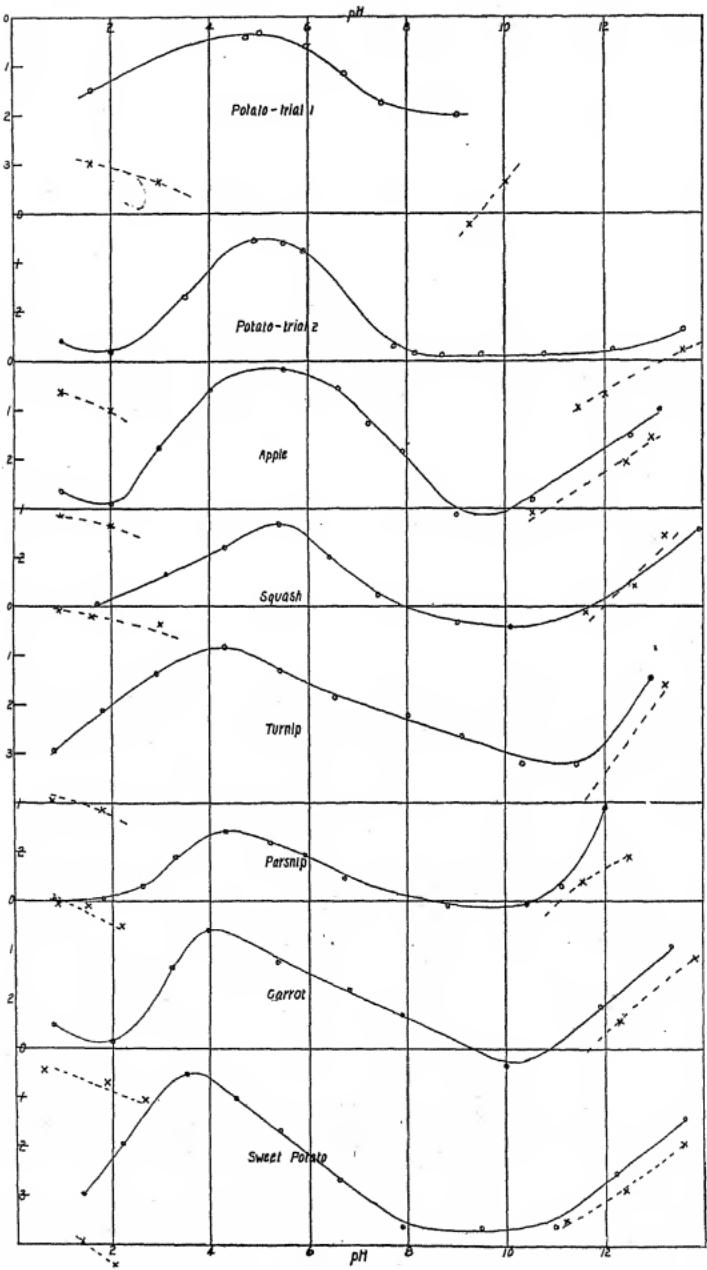


FIG. 1.—Rate of maceration of various tissues by pectinase at various hydrogen-ion concentrations; dotted lines show controls

The only possible conclusion that could be drawn from the results with *Sclerotinia cinerea* is that there is no intra- nor extra-cellular protopectinase present in the strains studied.

CONDITIONS FOR PRODUCTION AND ACTIVITY

After the failures already recorded to detect any protopectinase in *Sclerotinia cinerea*, this fungus was abandoned in favor of *Rhizopus tritici* for further study of the properties of the enzyme. A series of experiments was undertaken to determine the factors involved in its production and activity.

A careful study was made of various media to discover which would support a rapid and luxuriant growth of *Rhizopus tritici*. A liquid medium seemed more desirable because of its ease in preparing and handling, and also because of the possibility of testing for the extra-cellular enzyme.

HARTER and WEIMER (17) found that a decoction of sweet potato was very good for growing *Rhizopus tritici*. Decoctions of sweet potato, white potato, prune, and carrot were made and tested for their ability to support the growth of this fungus. The medium which proved the most efficient and practical was a decoction of carrots prepared by the following formula: To peeled carrots add double the weight of water, steam 4 hours, then filter through gauze and finally filter paper; add 25 cc. of this filtered carrot decoction to 250 cc. Erlenmeyer flasks, plug with cotton, and autoclave for 20 minutes at 15 pounds pressure. The resulting solution is clear and is found to be a very satisfactory medium for growing *R. tritici*.

Peach and prune media were made by soaking the dried fruit over night, then stewing for 2 hours. The juice was then pressed out, filtered, and diluted to the desired strength. Carrot juice supported the best growth, and was followed by sweet potato, white potato, prune, and peach juice respectively.

The acidity of the medium on which *Rhizopus tritici* grows does not affect the protopectinase production to any great extent (20). JONES found that an acid medium was more favorable for protopectinase production by *Bacillus carotovorus*. For spore production a solid medium was preferred. Soaked peach mash, potato agar, prune agar, sweet potato agar, and mashed carrot were all given a thorough

trial. Mashed carrots and potato agar produced a fine growth of spores and in a very short time; then in order of decreasing sporulation came prune agar, mashed potatoes, and mashed peach.

JONES found that the amount of enzyme in the medium surrounding *Bacillus carotovorus* increases with age to 19 days or longer. HARTER and WEIMER (17) found with *Rhizopus tritici* that the maximum enzyme content of the hyphae and of the medium is attained in about 24 and 48 hour old cultures. The hyphae were the most active, but the solution or medium upon which the fungus grew increased in activity up to 7 days or more.

TABLE IV
EFFECT OF METHOD OF GRINDING ON PROTOPECTINASE ACTIVITY

NO.	SAND (GM.)	TIME OF GRINDING (MINUTES)	TIME FOR MACERATION (MINUTES)
1.....	0.5	1	53
2.....	0.5	2	63
3.....	0.5	3	68
4.....	0.5	5	69
5.....	0.5	10
6.....	0	1	47
7.....	0	2	52
8.....	0	3	57
9.....	0	5	59
10.....	0	Shaking	68

Since enzymes are sometimes injured by too severe grinding, a comparison was made of the rate of maceration by mycelial hyphae ground in sand, and ground without sand. Table IV shows the results. In each case the concentration was 0.5 gm. of mycelial powder, 25 cc. water, and 0.5 gm. of sand when the latter was used. The results show that enzyme activity is decreased in both cases with the time of grinding. Grinding without sand produced a stronger mace- rating extract than grinding with sand, as the sand seemed to have a destructive action on the enzyme in every case. One minute of grinding without sand seemed to be the best way to obtain the enzyme extract, and this procedure was adopted. Filtering this extract through paper reduced its activity noticeably; therefore centrifuging was employed.

The time required to extract the enzyme from the ground myceli-

um was ascertained by testing the macerating power of the solution after standing for various periods of time in contact with the ground mycelium, using as before 0.5 gm. of the latter to 5 cc. of water, and grinding for one minute with toluene present. The extract was centrifuged and the macerating activity determined quantitatively on strips of potato, using the drop-weight method already described. Fig. 2 gives the results. The greatest strength of the extract was obtained in 4 days, but an extraction of 10-12 hours is shown by the curve to be the best for economy of time and consistency of results.

Results showed that after the extraction of the powder, centrifuging, and storing with toluene, the extract became weaker very gradu-

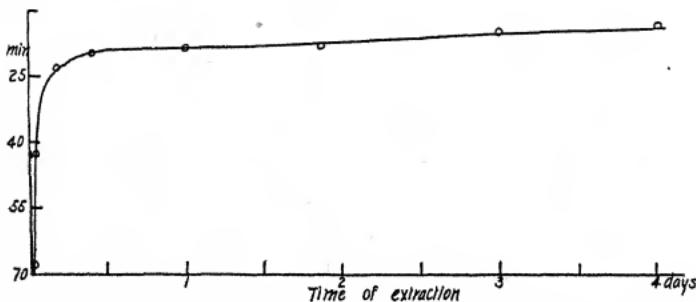


FIG. 2.—Relation between time of extraction of ground mycelium and protocellinase activity of extract.

ally; the activity decreased one-half or more in strength in 3 weeks' time. The dried and ground mycelium, kept at room temperature over concentrated sulphuric acid in a vacuum, lost about half of its activity in 5 months.

An enzyme extract was made as before, using 0.5 gm. of powder to 25 cc. of water. Successive maceration was carried out on strips of potato tissue, the strips being as nearly alike as could be made. Table V gives the results for three different periods of extraction of the mycelial powder. The first five macerations are very similar but noticeably decreasing in strength, until at the eighth maceration the activity is nearly gone. In short time extraction the strength is a function of the time of extraction.

Since every enzyme has a more or less definite optimum range of hydrogen-ion concentration, it seemed advisable to determine this for protopectinase. A series of solutions of varying hydrogen-ion concentration was prepared, therefore, and a number of different tissues used as testing material. The results are presented in fig. 1. The curves of the speed of maceration charted as the ordinates and the P_H as the abscissae show a slight decrease in maceration from P_H 1 to about 2, then a steady increase in maceration up to approximately P_H 5, followed by a corresponding regular decrease to from P_H 8 to 11, depending on the tissue used. Control determinations

TABLE V
EFFECT OF SUCCESSIVE MACERATIONS ON STRENGTH OF
PROTOPECTINASE SOLUTIONS

TRIAL	MACERATION TIME (MINUTES)	TRIAL	MACERATION TIME (MINUTES)
Extracted 24 hours			
1.....	21.5	1.....	28
2.....	22	2.....	57
3.....	22		
4.....	26		
5.....	26		
6.....	28		
7.....	36		
8.....	215	1.....	60
9.....	2.....	268
Extracted 1 hour			
Extracted 15 minutes			

with heated enzyme show a slow maceration at high acidities and high碱碱ities, but none in between. Each individual tissue shows its own characteristic curve, but the general shape of the curves is about the same for all tissues used, and indicates a well marked optimum at a P_H 4.5-5.

Toluol was used as an antiseptic. HARTER and WEIMER (17) found it very successful. It had no macerating effect on the common potato, and it did not retard protopectinase activity. The enzyme did not seem to be affected by the presence of the brass weights used in the determination of time of maceration. Light had no noticeable effect on its activity.

EXPERIMENTS WITH STANDARD PROTOPECTINASE

The maceration of tissues in the sense employed here means the separation of the cells from one another without the disintegration of the cells themselves. It is believed that the relative ease with which this maceration may be brought about is of importance in several ways, among which may be mentioned: (1) the relative susceptibility of tissues to invasion by those fungi which depend on protopectinase for penetrating the host tissue; (2) the firmness of various fleshy tissues, such as tubers and roots, after cooking; (3) the mealiness of potatoes; (4) the retting of flax. In making quantitative investigations of any of these phenomena, it would be highly desirable to have at hand a standard and very active preparation of protopectinase to be used as a reagent. In the following paragraphs are given a few preliminary results with the preparation and use of a standard solution of protopectinase, presented largely to show some of its possible uses.

With the foregoing facts at hand concerning the conditions for the production and use of this enzyme from *Rhizopus tritici*, it was possible to prepare a more or less standard solution of it for use as a quantitative reagent. The standard procedure was as follows: 1 kg. of thinly sliced carrots were boiled in 2 l. of water for 4 hours, filtered, and 25 cc. portions placed in 250 cc. Erlenmeyer flasks and sterilized. These were thickly sown with spores and incubated at 20° C. for 24 hours. The mycelial mats were removed from the flasks, rinsed, stirred in a large excess of acetone for 10 minutes, transferred to fresh acetone for 2 minutes and then to ether, after which they were dried by a fan and then over sulphuric acid in vacuum. A standard extract of this mycelium consisted of 0.5 gm. of it ground for 1 minute in 25 cc. of water containing 2 drops of toluene, and then centrifuged.

A series of comparative maceration experiments was run to discover how long it took the standard enzyme to macerate the various tissues. Ten cc. of the extract was placed in a number of tubes in a water bath at 37° C. Uniform strips of different tissue were cut and the time to macerate them estimated carefully, each determination being run in duplicate. Control strips in water did not macerate. Table VI shows the results of this experiment. A large range in time

of maceration is noted, varying from 2 minutes in pumpkin to 80 minutes in squash. Time was not available for studying the effect of maturity of tissue, position in plant, and other factors which suggest themselves.

TABLE VI

RELATIVE RATE OF MACERATION OF VARIOUS
TISSUES BY PROTOPECTINASE

TISSUE	TIME OF MACERATION (MINUTES)
Pumpkin	2, 2, 2
Sweet potato	34, 35
Carrot	41, 42
Potato	35, 34
Rutabaga	66, 63
Turnip	48, 48
Squash	80, 76

The size of the potato tuber and the region from which the test tissue is taken show a difference in rate of maceration. Table VII shows the data obtained on two varieties of tubers. The larger potatoes have a longer maceration period. The differences between cortex and medulla are irregular.

TABLE VII

EFFECT OF VARIETY, SIZE, AND REGION OF POTATO TUBER ON
RATE OF MACERATION

EXPERIMENT	VARIETY	SIZE	SECTION	TIME OF MACERATION (MINUTES)
1	Cobbler	Large	Cortex	37, 42
2		Large	Medulla	35, 35
3		Small	Cortex	23, 27
4		Small	Medulla	28, 30
5	Triumph	Large	Cortex	39, 36
6		Large	Medulla	56, 62
7		Large	Medulla	38, 37
8		Small	Cortex	37, 37

An attempt was made to correlate the mealiness of the potato with its rate of maceration. Tubers of uniform size were chosen, peeled, a piece removed from the cortex for a maceration test, and the remainder placed in 1000 cc. beakers and 600 cc. of boiling water added. The potato was boiled until done, as shown by testing with a fork. The mealiness was tested as soon as the potato was removed

from the water by breaking it open with a fork and noting its appearance. The extent of sluffing was estimated by the general appearance of the tuber and the amount of potato particles that were lying in the beaker after the cooking test was completed.

Table VIII shows that there was no regular relation between mealiness, sluffing, and time of maceration. It was thought that the rate of maceration might vary with mealiness, but no regular indication of this could be seen.

TABLE VIII
MEALINESS, SLUFFING, AND RATE OF MACERATION OF DIFFERENT
VARIETIES OF POTATO

VARIETY	MEALINESS	SLUFFING	RATE OF MACERATION
Cobbler.....	++++*	+++	28, 32
Cobbler.....	+++	++	20, 21
Triumph.....	+++	+	38, 36
Triumph.....	+++	+	36, 36
Triumph.....	+++	++	24, 24
Triumph.....	+++	+++	32, 31
Triumph.....	+	+++	25, 27
Triumph.....	+	+++	25, 26
Triumph.....	++	+	27, 29
Triumph.....	+++	+	25, 29
Cobbler.....	+++	++	30, 31

* +++ very mealy; +++ great deal of sluffing.

MACERATING POWER OF OXALATES

The question as to the macerating ability of oxalates or oxalic acid has been much discussed, and there is little agreement. COOLEY (12) found it present in rotted peaches and plums, but was doubtful whether it was a middle lamella solvent. VALLEAU showed that oxalic acid would soften plum and peach but not potato tissue. SMITH (33) compared the mycelial extract of *Botrytis* with weak solutions of oxalic acid on stems of lettuce, and found that the oxalic acid alone induced a macerating action on the tissue similar to that of the extract of the fungus material, and on the basis of these results came to the conclusion that the dissolution of cell walls noted is due to the oxalic acid secreted by the fungus.

Several common oxalates likely to be found in plants were chosen and used at a concentration normally possible in the tissues. The P_n's were adjusted as shown in table IX, and the maceration noted.

A blank series without oxalate was also run. The plant tissues selected were the potato, apple, lemon, and carrot. Table IX shows that sodium, potassium, and ammonium oxalate have little if any effect on the tissues used. The control shows that an alkalinity of 10 to 11 had a slight macerating effect, while a P_H as low as 1.55 had no effect. Apple tissue seemed slightly macerated by the oxalates, but this effect may be only a slight hydrolysis or softening due to water present.

TABLE IX
MACERATION OF TISSUES BY OXALATES

P_H	POTATO	APPLE	LEMON	CARROT
2.80.....	---	?	---	---
4.80.....	---	---	---	---
5.52.....	---	---	---	---
6.46.....	---	---	---	---
8.58.....	---	---	---	---
2.10.....	---	---	---	---
4.50.....	---	---	---	---
4.90.....	---	---	---	---
6.80.....	---	---	---	---
9.58.....	---	---	---	---
1.65.....	---	---	---	---
3.65.....	---	---	---	---
5.35.....	---	---	---	---
7.15.....	---	---	---	---
10.02.....	---	---	---	Slight
1.55.....	---	---	---	---
3.68.....	---	---	---	---
7.14.....	---	---	---	---
10.20.....	---	---	---	Blank
11.00.....	---	---	---	Slight

IV. Pectase

SOURCE AND PREPARATION

This enzyme, which coagulates soluble pectin to a gel, was discovered by FREMY (16) in 1840. He prepared it by the alcoholic precipitation of the juices of several plants.

No thorough search was made for this enzyme. Table I shows the substances which were tested and the results obtained. Corn pollen, clover leaves, and *Sclerotinia cinerea* were the only materials which contained the enzyme.

In the following studies the juice of clover and an extract of corn pollen proved abundant sources for pectase. This was prepared from the clover by first pressing out the juice in a hydraulic press, centrifuging, adding a little chloroform, and setting it away from the light. After 24 hours a coagulum formed which could be filtered. The filtrate was dried by an electric fan, then over sulphuric acid in a vacuum. The dried powder was ground and screened.

Corn pollen was extracted with water for several hours, then centrifuged and was ready for use. A powder of very strong enzyme activity was prepared from this pollen extract by precipitating with two volumes of alcohol, filtering, drying, and powdering.

When *Sclerotinia cinerea* grew on prune juice it secreted pectase, which was indicated by the formation of a flocculent gel in the medium below the mycelium. The enzyme could be precipitated by alcohol from the prune juice in which the fungus had grown.

DETERMINATION

It was found very difficult to develop a quantitative method for pectase estimation, but a qualitative method is relatively simple. Twenty cc. of a 1 per cent solution of lemon pectin⁴ is placed in a 50 cc. Erlenmeyer flask, a pinch of CaCO₃ added, and mixed well. The determination is carried out at room temperature. An extract of the material to be tested is added to the flask and the time of coagulation and general consistency of the gel noted. The time of appearance of syneresis is also noted, and the general relation between time and consistency used in judging the relative pectase activity. The burden of the determination is left to the individual judgment, which makes it very approximate quantitatively, but easy to detect qualitatively.

CONDITIONS FOR PRODUCTION AND ACTIVITY

Very little has been done with regard to the conditions best suited for pectase activity. It is known that calcium, barium, or strontium must be present in the solution for gelation to occur (25), and EULER and SVANBERG (15) determined its optimum P_H to be at about 4.3.

⁴The pectin was kindly furnished by Mr. C. P. WILSON of the California Fruit Growers Exchange.

The method used for studying the effect of the hydrogen-ion concentration on pectase activity was as follows. Twenty cc. of 1 per cent lemon pectin was placed in a series of 50 cc. Erlenmeyer flasks and acid and alkali added to bring about the desired reaction. Then 10 cc. of active pectase was added and the P_H determined at once by the potentiometric method. Any change in volume due to adding an acid or alkali was corrected for. A control was run in the same man-

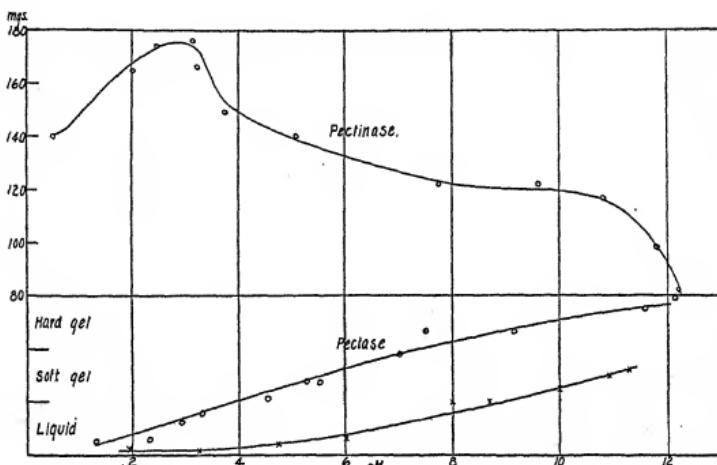


FIG. 3.—Relation between hydrogen-ion concentration and activity of pectinase and pectase; crosses show controls.

ner as the original, using a 1 per cent pectin solution at the different P_H 's, but with no pectase present. Fig. 3 shows the results. There was a gradual increase in viscosity as the acidity decreased. Production of a firm gel began at a P_H of 7. In the control a soft gel begins to form at this point. From these data no optimum point or even zone can be detected. From the experiment it is clearly shown that alkalinity alone will produce a soft gel, beginning at about P_H 8, but when combined with pectase produces a firm gel. On the extreme acid side no pectase activity is apparent.

All observations indicate that pectase is very stable. No change in the activity of the dried precipitates was detectable up to 4 months. The extracts were not kept so long, due to contamination,

but were very active until contaminated. The pollen grain powder which had been stored for 6-8 years was still very active. Other pectic enzymes may have been present at one time, but pectase was the only one present after this period.

Filtering through paper did not remove any of the enzyme, but centrifuging was finally adopted, for uniformity with the treatment used for the other pectic enzymes.

V. Pectinase

SOURCES AND PREPARATION

Table I shows the results of running pectinase tests on the same series of materials as used for the other pectic enzymes. Ptyalin, amylopsin, and several of the other enzymes from animal origin were tested, but no pectic enzyme was found, and hence in a way they acted as a control on the methods used.

The best sources of pectinase were *Rhizopus tritici*, *Sclerotinia cinerea*, and *Botrytis cinerea*. In all cases the enzyme could be prepared by grinding the material with water for several hours, then precipitating the filtered extract with alcohol.

A mycelial powder was made by inoculating sterile flasks of prune juice or carrot decoction with *Sclerotinia cinerea* and *Rhizopus tritici* respectively, and incubating at 21° C. for 24-48 hours, then harvesting, washing, and drying by Dox's method. From this dried powder a standard extract was made by mixing 0.5 gm. of the powder with 25 cc. of H₂O, and leaving for 10-14 hours. After centrifuging, the extract was ready for use. The medium upon which the *Rhizopus* had grown for several days was also a good source of pectinase. It may be used as it occurs in the liquid medium, or precipitated with two volumes of alcohol.

DETERMINATION

A pectinase determination is carried out by estimating the reducing substances formed by the action of the enzyme on pure pectin. Pectinase hydrolyzes soluble pectin, and also the gel produced by the action of pectase on pectin, to reducing substances, probably arabinose and galactose (14). To 20 cc. of 3 per cent pectin is added 10 cc. of enzyme extract, made acid with HCl, and placed in an incubator at

40° C. for 24 hours. A control is made by omitting the pectin. The pectin used was found to have no reducing action. After hydrolyzing the solution was clarified by one of the common methods, and reducing sugars run by the picric acid method (41). By correcting for the control and calculating the reducing substances as galactose, an accurate but comparative indication of pectinase content was obtained.

CONDITIONS FOR PRODUCTION AND ACTIVITY

The fungi in question produced pectinase on the same media on which they produced the other enzymes. The age of the culture for maximum pectinase production was found to be somewhat older than that found for maximum protopectinase activity. A 48-hour culture was used, but there were indications that an older culture would produce a stronger pectinase reaction.

Pectinase was found to be very stable, judging from observations made on the activity of extracts kept for months under toluene. The dried alcoholic precipitations of pectinase were also rather stable, as well as the dried powdered mycelium when kept over concentrated sulphuric acid in a vacuum. The activity of pectinase was somewhat impaired by filtering, so centrifuging was strictly adhered to.

The concentration of pectin on which the pectinase extract acted had considerable effect on the amount of reducing substances formed. Solutions of pectin of 0.5-5 per cent concentration and enzyme extract were mixed together and set aside for 24 hours, and reducing activity was determined. The results were irregular, but indicated in general the same fact as for most enzymes, that increase in concentration of substrate increases the work done per unit of enzyme, but the relation is not linear. The data are presented in fig. 4.

VI. Existence of three pectic enzymes

As was stated in the introduction, some writers assume but two pectic enzymes, on the basis that the same enzyme causes both a maceration of plant tissue and a hydrolysis of soluble pectin. One of the main objects of the present study was to secure decisive information on this question. In this section are collected the main facts at hand bearing on the identity of these enzymes, including not only the facts previously discussed, but also evidence adduced to show

that there are three separate enzymes. For the sake of clarity a scheme is presented in table X which shows the composition of the pectic substances, so far as this is now generally accepted, and the relations of three possible enzymes to these substances. The facts that bear on the identity of the pectic enzymes, and which substantiate the existence of three of them follow.

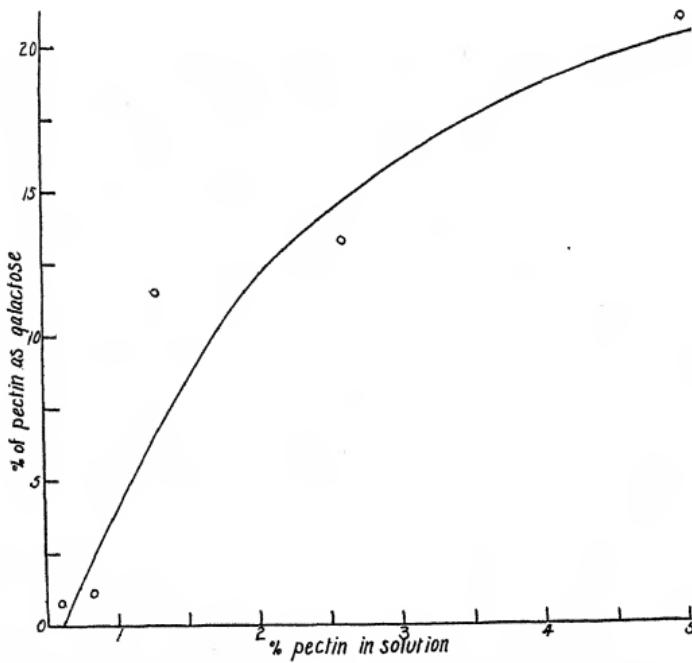
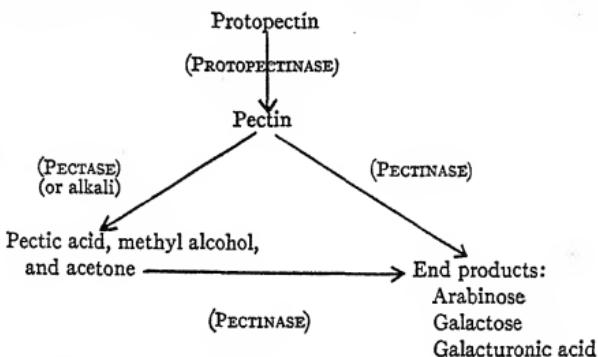


FIG. 4.—Relation between pectinase activity and amount of substrate present

One and possibly the only method for the identification of enzymes is by the nature of their end products, and the changes they produce. Table X gives the relation of the pectic enzymes to one another and to their end products. TUTIN (35), studying the effect of alkalies and of pectase on pectin, found methyl alcohol, acetone, and a salt of pectic acid as the decomposition products. He concludes that pectin is probably the dimethylisopropenyl ester of pectic

acid. TUTIN does not believe that pectin exists in the plant as pectose or protopectin. This CARRÉ (11) confutes, claiming on the basis of chemical and microscopic evidence the presence of an insoluble pectic constituent in apple tissue called pectose, which is converted by hydrolysis to pectin. CARRÉ believes that the long extraction carried out by TUTIN caused hydrolysis.

TABLE X
RELATIONS AMONG PECTIC SUBSTANCES AND THEIR ENZYMES



The writers repeated the work of TUTIN. A 1 per cent solution of lemon pectin was treated in a cold aqueous solution with 13.4 per cent of its weight of NaOH (the amount neutralized by pectin in the cold), made slightly acid, and fractionally distilled. The SCUDDER and the SCUDDER and BRIGGS tests (32) were used for methyl alcohol, while the LIEBEN iodoform test (38) was used for acetone. A test for both methyl alcohol and acetone was obtained with the methods used. This corroboration of TUTIN's results indicates that pectase must be an esterase; and since there is no evidence that ester linkages are involved in any of the other pectin hydrolyses, pectase must be different from protopectinase and pectinase. The specific nature of the maceration action on tissue and of the production of sugars from pectin makes the last two enzymes appear quite distinct.

Since the differences in temperature of inactivation may form a basis of separating enzymes, and hence of proving a lack of identity among them, this point was determined for the three enzymes under

discussion. Each was prepared according to the methods outlined, and then the enzyme solutions in test tubes were exposed for 20 minutes to various temperatures. They were then cooled, and the

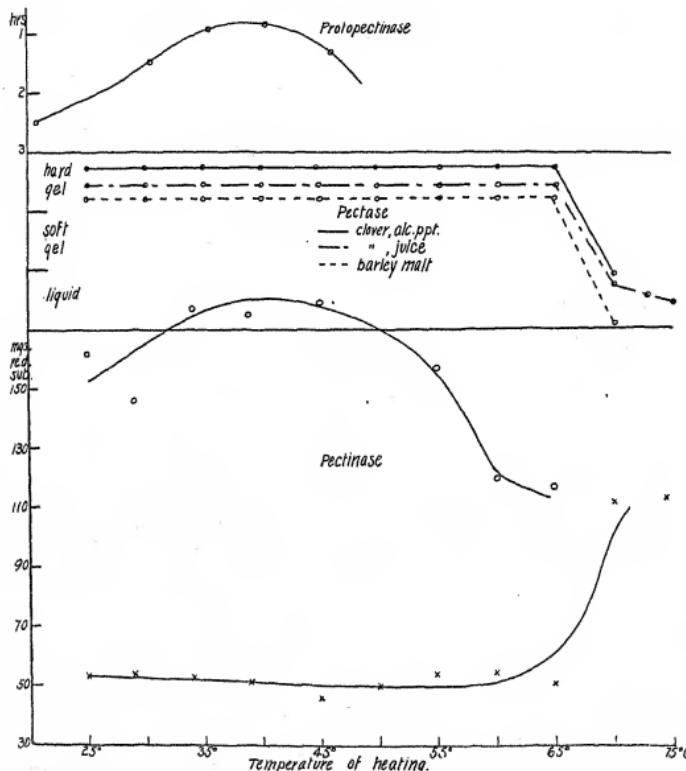


FIG. 5.—Temperature of inactivation of pectic enzymes; crosses show control

activity measured at 20° C. in the usual way. The results are shown in fig. 5.

It will be seen that the temperature of inactivation of protopectinase is rather sharp at about 48° C., that of pectinase rather vague at about 60° C., and that of pectase at about 68°–70° C. The first figure agrees fairly well with that of 51° C. found by JONES (24).

This variation of temperature in inactivating the enzyme made it possible to use this as a method of separation and identification. This was performed successfully in the case of *Rhizopus tritici* extract, which contains protopectinase and pectinase. Again, the three enzymes were mixed and their activities checked successively by heating the enzymes to their thermal death points, first eliminating the protopectinase, then the pectinase, and finally the pectase.

The P_H of a medium in which an enzyme acts has much to do with the rate of its activity. Figs. 1 and 3 show the effect of the hydrogen-ion concentration on the activity of these enzymes. The optimum reaction for protopectinase is P_H 5, and of pectinase 3; that of pectase is indefinite, but is greater than 7. These differences in

TABLE XI
FRACTIONAL ALCOHOLIC PRECIPITATION OF MIXTURE OF THE THREE ENZYMES

NO. OF FRACTION	ALCOHOL (PER CENT)	PRECIPITA- TION	PROTOPEC- TINASE	PECTINASE	PECTASE
1.....	25	Medium	++++	-	-
2.....	50	Light	++	+	-
3.....	85	Medium	++	++	-
4.....	90	Light	+	+++	-
5.....	95-98	Light	+	-	+++

optimum reaction are significant and appreciable, and should be of service in distinguishing the three enzymes.

The sources of the various enzymes have been studied to some extent. Table I gives a list of materials tested for pectic enzymes in the present work. In no case so far have all three enzymes been found together. It would be futile to attempt an explanation of this at present. It is a strong argument, however, in favor of the non-identity of the three enzymes.

Alcoholic precipitation was attempted in the separation of the pectic enzymes. A fractional precipitation was tried on a mixture of *Rhizopus tritici* extract and clover juice. The method adopted was to add alcohol until a precipitate was noticed, filter after 3 hours of standing, and then increase the percentage of alcohol until another precipitate was recognized. Table XI gives the results. It will be seen that protopectinase activity decreases with the percentage of alcohol used, and with successive precipitates. At the same time the

pectinase and pectase activities increased. These results also favor the view that there are three pectic enzymes.

A study was made of the adsorption of these enzymes under varying conditions on various adsorption reagents, as kaolin, charcoal, fullers' earth, and aluminum hydroxide. LEVENE and WEBER (27) used this method for the purification of enzymes with some success. HEDIN (23) also had success by the differential adsorption of enzymes on charcoal and kieselguhr, the charcoal removing beta-protease, and the kieselguhr alpha-protease.

Adsorption of protopectinase on Lloyd's reagent (hydrated aluminum silicate) was attempted. Thirty gm. of the reagent was added to 1000 cc. of carrot medium upon which *Rhizopus tritici* had grown for two days, and the mixture shaken every few hours. The reagent removed protopectinase from the solution quantitatively. The reagent with the adsorbed enzyme was then placed in a series of solutions of varying P_H , using 3, 6, 7, 8, and 12, in the attempt to free the enzyme again, but unsuccessfully. Kaolin was next used as an adsorption agent. A 3 per cent mixture of kaolin and protopectinase extract was made and left for 14 hours with occasional shaking. The kaolin removed the enzyme from solution, but again treatment of the adsorption complex with a range of solutions of P_H 3-9 failed to remove the enzyme. Norit was also tried in the same concentration and manner of treatment as kaolin, and negative results were again obtained.

The pectinase used in these experiments was prepared from mycelia of *Sclerotinia cinerea* and *Rhizopus tritici*, and from the carrot medium upon which the latter had grown. Charcoal (norit), Lloyd's reagent, and kaolin were used to adsorb the enzyme from each of the solutions, in the following manner. Thirty gm. of the adsorption reagent was added to 1000 cc. of the enzyme extract or solution upon which *Rhizopus tritici* had grown for 2 days. The mixture was shaken occasionally during a period of 15 hours, then centrifuged, and the solid dried by a fan and then over sulphuric acid in a vacuum. Lloyd's reagent removed the pectinase quantitatively from the extract, but kaolin and norit were not quite so efficient. The enzyme could not be freed from these materials by digesting in solutions, the P_H 's of which varied from 4 to 9.

A strong pectase was prepared by extracting corn pollen with water for 10 hours and centrifuging the liquid. Kaolin, Lloyd's reagent, and norit were again used for adsorbing the enzyme. The first two reagents removed the enzyme completely, but the norit only incompletely. By varying the P_H between a range of 4 to 9, no pectase activity could be recovered from these adsorption complexes. From the Lloyd's reagent a slight activity was recovered at P_H 11.6, but as the control showed also a slight gelling, the enzyme action is doubtful. Table XII shows the results of these adsorption studies in a more collected form. They were not successful in showing any differences among the three enzymes.

TABLE XII
ADSORPTION OF PECTIC ENZYMES

ENZYME	ADSORPTION REAGENT	ADSORPTION	LIBERATION
Protopectinase...	Lloyd's reagent..	Complete	None
	Kaolin	Complete	None
	Norit	Complete	None
Pectinase.....	Lloyd's reagent..	Incomplete	None
	Kaolin	Incomplete	None
	Norit	Incomplete	None

It was noticed that the protopectinase activity varied with the method used, whether the enzymes were removed from the débris of cells by filtering or centrifuging. For that reason the effect of filtering and centrifuging was carefully tried out on the three pectic enzymes. Table XIII gives the results of the experiment, which might be summed up by saying that filtering decreases only protopectinase activity. Nevertheless centrifuging was adopted for all three enzymes.

Practically all of these criteria concerning the existence of these pectic enzymes indicated that there are three. From what information is available now concerning the composition of the pectins, and the occurrence and function of these enzymes, the following generalizations may be offered. Protopectinase macerates the tissues, forming pectin, thus making it possible for the mycelia of the fungi to penetrate that tissue. Then pectinase acts on the pectin and hydrolyzes it to the simpler end products, and these serve as food for the

fungi. Pectase coagulates soluble pectin. What its usefulness may be is not known at present. WILLAMAN (40) suggested that in the case of *Sclerotinia cinerea* the gel formed conveys hydrophylic prop-

TABLE XIII
EFFECT OF FILTERING AND CENTRIFUGING ON ENZYMES

ENZYME	SUBSTRATE	ENZYME TREATMENT	RESULTS
Pectase	10 cc. pectin, 1 per cent CaCO_3	(Centrifuged First filtration Second filtration Third filtration Fourth filtration)	Hard gel
Protopectinase	Potato tissue.....	(Centrifuged First filtration)	Maceration time 18 minutes Maceration time 25 minutes Mg. reducing substance as glucose
Pectinase	10 cc. of 3 per cent pectin.....	(Centrifuged First filtration Second filtration Third filtration Fourth filtration)	498 501 510 525 515

erties on the plum tissues that are advantageous to the fungus in the "mummy" stage of the parasitism. Some of the properties of the pectic enzymes are summarized in table XIV, so that the factors discussed can more easily be compared.

TABLE XIV
SUMMARY OF PROPERTIES OF PECTIC ENZYMES

	PROTOPECTINASE	PECTASE	PECTINASE
Substrate.....	Protopectin	Pectin	Pectin and pectic acid
Gross effect of action.....	Maceration of plant tissue	Coagulation Ester	Hydrolysis Ether (glucosidic)
Type of linkage attacked..	Unknown		
Point of thermal inactivation.....	48° C.	68°-70° C.	60° C.
Optimum P_H	5	Above 7	3-6

Summary and conclusions

1. Protopectinase (pectosinase) has been prepared from *Rhizopus tritici* and *Bacillus carotovorus*; in the former both the intracellular and extracellular, and in the latter only the extracellular form was used.

2. Pectase has been prepared from clover leaf sap, corn pollen, and the mycelium of *Sclerotinia cinerea*.
3. Pectinase has been prepared from the mycelia of *Rhizopus tritici*, *Sclerotinia cinerea*, and *Botrytis cinerea*, and from barley malt.
4. Quantitative methods for measuring the activities of these enzymes have been developed, and the conditions for their production and action determined.
5. A careful search among many cultures of *Sclerotinia* and related forms failed to disclose protopectinase activity in any of them.
6. The rate of maceration of several tissues, including large and small potato tubers, different regions of the tubers, carrot, squash, sweet potato, pumpkin, turnip, and apple was measured.
7. Convincing evidence was obtained in support of the view that there are at least three distinct pectic enzymes, namely, protopectinase, pectase, and pectinase. The lines of evidence are (1) all three enzymes have not been found in the same plant material; (2) they have quite different temperatures of inactivation; (3) they differ somewhat in their optimum P_{H_2} ; (4) they can be separated more or less completely by alcoholic precipitation; (5) protopectinase is appreciably adsorbed onto filter paper, while the others are not; (6) pectase probably hydrolyzes ester linkages, while the others hydrolyze other linkages.
8. Sodium, potassium, and ammonium oxalates were found not to macerate tissues of lemon, potato, carrot, and apple, over a wide range of hydrogen-ion concentration.

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ALTERNATION OF GENERATIONS IN RELATION TO REDUCTION DIVISION

NILS SVEDELIUS

The problem of the alternation of generations of late has come once more to the front in botanical discussions. This probably is due to the fact that our knowledge of the life history of the lower plants, and especially the cytological side of it, has undergone a very important extension. The significance of reduction division in marking limits of the generations has become a hotly contested question. Opinions differ as to whether the alternation of generations established by HOFMEISTER (22) really has any connection with the alternation of haploid and diploid states of the nuclei. A survey of the various opinions that have been expressed on this question, and of the terminology used by the disputants, leads one to agree with the pronouncement of BRAUN (3), made as far back as 1875, that "generation is really an elastic conception."

The introduction of the cytological element into the problem of alternation of generations, by STRASBURGER (40), gradually but consistently brought us to the point where the concept "antithetic alternation of generations" became synonymous with alternation of haploid and diploid states of the nuclei. The reaction against this conception from BUDER (4), KYLIN (30), and RENNER (36), who regard generations from a purely morphological point of view, has led to the surrender of the antithetical conception of the alternation of generations, with the result that the life cycle of a plant can be divided into any number of generations. Consequently, one still finds in many modern works on the alternation of generations the conception which was current as early as 1860. The explanation of this, of course, is the difficulty, not to say impossibility, of fixing the meaning of the term "generation." The adherents of the purely morphological view find their support in a certain principle of priority, since all cytological aspects of the problem were beyond the purview of HOFMEISTER; indeed, they were at that time unknown. On the contrary, a sharp line between external and internal morphology (anatomy and cytology) cannot easily be drawn; therefore

the attempt to characterize the generations by cytological marks cannot be stamped as incorrect (BUDER 4), because the generations were originally distinguished by purely morphological characters. Laws of priority are impossible here, they may be employed in giving plants and animals scientific names, but the history of science shows that the principle of priority cannot hold good in the sphere of general terminology. Language is more conservative than thought, and words live on and continue to be used though their meaning may have changed more than once. We have only to think of the concepts "cell," "fertilization," "phanerogam," "cryptogam," etc. Our modern conception of cell is far removed from that which ROBERT HOOKE denoted by this name in 1667; that is, we still use the word but put a different meaning into it.

The idea of "fertilization" cannot be explained nowadays without reference to the cytological conditions, although the conception "foecundatio" with reference to plants is met with as early as in the works of CAMERARIUS, KOELREUTER, and LINNAEUS. What they meant, however, was probably about the same as we now mean by "pollination." The word fertilization, of course, has never been altered, but the conception now indicated by this term includes much more than the mere transference of pollen grains to the stigma. The same thing has happened to the expression "alternation of generations." In the course of time this, too, has been very considerably changed in meaning. HOFMEISTER'S (22) conception is a very different thing from what CHAMISSO and STEENSTRUP (39) had much earlier called by this name, on account of their discoveries concerning the evolution of the Salpae, Medusae, and Trematodes. Now, however, instead of first insisting upon a definite conception of alternation of generations, it is held to be more important to show whether there exists a universal and common explanation of, or a biological connection between the different phenomena which have, by various authors at different times, been designated as "antithetic" and "homologous," or "morphological" and "cytological" alternation of generations. Only after such an investigation can we gain a clue to the explanation of the remarkable phenomenon which recurs in widely separated groups of plants, in each of which the life cycle can be divided into rhythmically recurring phases or sections

that sometimes become separate individuals. These phases are sometimes somewhat alike; on the other hand they sometimes differ considerably in degree of development; in other words, I mean that singular relation of alternation in the ontogenetic development of plants which NÄGELI (32) called "one of the most remarkable phenomena of the plant-world."

Could all these types of alternation be shown to be variants of the same phenomenon, then the solution of the much contested problem of alternation of generations might be said to have been begun. To this solution I shall now endeavor to make a contribution.

It is characteristic of the first observers of the alternation of generations in both animals and plants that, as a rule, they make no attempt to explain this phenomenon. CHAMISSO, STEENSTRUP, and HOFMEISTER all content themselves merely with establishing the fact itself. The discoveries were themselves of such a sensational kind that the establishment of the fact itself sufficed and left no need of the addition of an explanation, which perchance could not then have been given. ČELAKOVSKÝ (6) states:

It is a perceptible fault of most presentations of the alternation of generations that the actual phenomena are simply registered without any effort being made to explain the alternation of generations, so that it remains an astonishing but incomprehensible fact.

Very soon, however, attempts at explanation were forthcoming. For instance, the zoologist LEUCKART thinks that the alternation of generations is a sort of division of labor between food preparation and reproduction. Something of this kind may perhaps have been in the thought of HOFMEISTER (22), when he remarked: "Vegetative life in mosses is exclusively of the first generation, fruit formation of the second. In ferns the case is almost the reverse."

The problem of the significance of alternation of generations is intimately connected with that of the origin of the generations; for, generally speaking, if a biological explanation of the significance or work of any particular organization is sought, it is necessary to know how this organization arose. In other words, a knowledge of its phylogenetic origin is the first condition for discovering the biological significance of any organization.

The first attempts at explanation of the alternation of generations were altogether on the lines of the Darwinian theory of evolution. The alternation of generations was regarded as being similar to the metamorphosis of the individual organ. The first generation, the "protophyte" of CĚLAKOVSKÝ (6), was followed by the second, the so-called "antiphyte," in the same manner that a higher stage of evolution follows a more primitive one in the development of an individual organ. Such an explanation, however, does not really touch the kernel of the problem.

Other attempts to solve the problem more from the biological side were made for instance by NÄGELI, who thought the alternation of generations was *inter alia* an "adaptation to the alternation of the seasons," so that at the end of a vegetation period another generation arises which is better adapted to the new surroundings. NÄGELI admits, however, that he has not hereby solved the problem. Thus he says at the close:

This fact of the alternation between the two generations to which the ontogenesis of the main order of the vegetable kingdom is due is one of the most remarkable phenomena of phylogenetic evolution. For the causal explanation of this fact of alternation I am unable to suggest anything but the struggle for differentiation.

To enter into all the numerous attempts at an explanation of the alternation of generation is of course impossible. I shall therefore only examine the most important explanation, namely, the hypothesis offered by BOWER (1, 2), which was later adopted by WETTSTEIN. This may be termed a migration theory, because the alternation of generations is looked upon as evolved in connection with the migration of plants to land, whereby the higher orders of plants represent "so many steps of the great process of the adaptation of a series of plants, which were originally purely aquatic, to a terrestrial life" (WETTSTEIN 50). In the same measure that plants became more and more decidedly terrestrial in habit, the sporophyte became more and more dominant, while the gametophyte, which was better adapted to an aquatic life, suffered reduction. This hypothesis finds its chief support in the circumstance that within the series moss-fern-seed plants the sporophyte proves to be organized more for land life than the gametophyte, the organization of which shows

it to be well adapted to aquatic life, and the fertilization of which by means of spermatozoids is absolutely dependent on water. This migration theory was further combined by BOWER (2) with a purely morphological theory to explain the rise of the sporophytes, the so-called interpolation theory. The sporophyte is here regarded as a new formation, arisen and developed from the zygote, which has gone on its own way of evolution. The sporophyte, as it were, is interpolated between two gametophytic generations, and the considerable difference between the generations is naturally explained by regarding the sporophyte as a new formation adapted to terrestrial life, while the gametophyte still remains a more or less pronounced water plant, which still preserves the peculiarities of organization acquired during its aquatic period. This interpolated organism is supposed by BOWER (2) to proceed in such wise that the sporogenous cells, which were originally the only ones formed from the fertilized ovum or zygote, are transformed by "sterilization" into a vegetative, food making system. Finally these vegetative tissues increase more and more, the sporogenous tissue becoming more and more limited and localized; lastly there appear definite accessory organs of various functions; for instance, leaves for the preparation of food, organs for the exposure of the sporangia, etc. The theory of interpolation and sterilization was then worked out in detail by BOWER (2). His theory gained wide acceptance; yet it must be emphasized that numerous investigators have been critical toward the ideas of interpolation and sterilization, which, in this theory, have come to be regarded as antithetical. PRINGSHEIM (34), by his discovery of apospory in mosses (the production of protonemata directly from the seta), soon after FARLOW's (15) discovery of apogamy in ferns, came to the conclusion that the alternating generations "also possess a homogeneous morphological character," that is, they are homologous. PRINGSHEIM (35) became thereby the founder of the theory of the homologous alternation of generations, which is now opposed to the antithetical theory of CĚLAKOVSKÝ and BOWER. In full agreement with this conclusion, PRINGSHEIM assumed that the sporangia, antheridia, and archegonia (or oogonia) were all homologous. These two theories of alternation, the so-called anti-

thetical and the homologous, have thus been irreconcilably opposed to each other.

The antithetical theory gained increasing support, and the biological explanation of the alternation of generations as a migration theory has still further contributed to its establishment. Still more important aid to this end, however, was furnished by the great cytological discovery that the essential feature of fertilization is a conjugation or fusion of nuclei followed by reduction division. This purely cytological school, whose most eminent representative was STRASBURGER, has still further strengthened the position of the antithetic theory, by showing that the cytological cardinal points, the fusion of nuclei and the reduction division, strictly coincide with the limits of the two generations determined by HOFMEISTER on quite different morphological grounds.

There is of course a great deal in the antithetic migration theory which is undoubtedly attractive. The fitness of the gametophyte for an aquatic life is as undeniable as is that of the sporophyte for terrestrial life. The question arises, however, whether this theory still holds good in view of recent discoveries regarding the history of development of the lower plants, discoveries which have been made since the theory was first propounded. The theory found justification as long as the alternation of generations of higher plants (mosses, ferns, and phanerogams) was the only one known. The discovery of a like alternation of generations in the algae, which have never migrated to land, must arouse suspicions; therefore the origin of the alternation of generations is not explained biologically by the theory of migration from water to land.

The first discovered cases of alternation of generations appeared to support the migration theory, to some extent at least, since in *Dictyota* both generations (except for the number of chromosomes and the reproductive organs) are exactly alike. In this case, also, we had to do with a plant both of whose generations lived under identical external circumstances. This discovery of the alternation of generations in *Dictyota* by WILLIAMS (51 52) and HOYT (23) aroused great interest. STRASBURGER (42) finds *Dictyota* "remarkable" from the standpoint of the alternation of generations, as the

generations are alike, and says this has now become a problem to whose solution we must apply ourselves. In this connection it may be of interest to mention that when NÄGELI (32) in 1884 discussed the alternation of generations, suspected even then, between sexual individuals and tetrasporic individuals in the red algae, he adduced as one of the weightiest arguments against this view the fact that the supposed generations were so similar. The knowledge which had been obtained as to alternation in mosses, ferns, and phanerogams exercised such an influence on opinion regarding the alternation of generations, that NÄGELI declared outright that it was inherent in the nature of generations to be different.

Meanwhile our knowledge of the life history of the lower plants was extended more and more. As early as 1907 YAMANOUCHI (53) showed that the reduction division in *Polysiphonia* takes place at the division of the tetraspore mother cell, and that the red algae afford an instance of a regular alternation of generations between a haploid generation with male or female organs, and a diploid, spore forming generation. A noteworthy complication here is the diploid nature of the cystocarp. That is, the morphologically distinct generations do not fully coincide with the phases of development distinguishable by the single and double chromosome numbers. The conditions found in *Polysiphonia*, as later investigations by LEWIS (31), SVEDELius (43, 44), and KYLIN (26) have shown, recur in *Griffithsia*, *Delesseria*, *Nitophyllum*, *Rhodomela*, etc., and in all probability in the majority of the Florideae. Meanwhile there arose an interesting question regarding those Florideae which never form tetraspores, for there are many such. The writer (45) succeeded in finding in *Scinaia* a type that gave an answer to this question. In this genus the reduction division occurs immediately after fertilization. The same thing occurs, as was shown later by KYLIN (28, 29), in other Florideae belonging to the Nemalionales (*Nemalion*, *Batrachospermum*, and probably in all other Nemalionales). How shall these differences in a group which is incontestably uniform phylogenetically be explained? In my opinion (45-48) these different types can only be accounted for on the supposition that in the course of their evolution there has been a postponement in the time of occurrence of the reduction division. In the undoubtedly primitive *Scinaia*

type, the reduction division occurs immediately after the nuclear fusion. In other derived forms the reduction division for some reason has been dropped at this point, and thus the whole cystocarp has become diploid. The diploid carpospores thus formed germinate and give rise to diploid plants, which form no sexual organs. Reduction division occurs in their monosporangia, however, so-called tetraspores being formed, at whose germination plants arise which are able to carry out the formation of male and female organs. It is thus, in my opinion, that all at once a diploid generation may arise. I am unable to say what is the cause of this delay of the reduction division. Possibly hormones may play some regulating part; a field of work is here open to the physiologist. An attempt must be made by variation of the external conditions to influence reduction division in such wise that it is either postponed or compelled to occur earlier.

The fact should be emphasized that by the delay of reduction division in the Florideae, in the manner described, a new generation (the tetrasporic plant) arises at one stroke. It has not arisen by a progressive sterilization, as the interpolation theory of BOWER (2) assumes, but suddenly.

This assertion, however, does not exclude the possibility of really interpolated organizations likewise occurring. The Floridean cystocarp, for example, arose, in the writer's opinion, by the later formation of sporogenous filaments from the zygote by "sterilization," whereas the zygote in more primitive forms was merged into spore formation, as still happens in the Bangiaceae. One may be fully justified in speaking in the spirit of BOWER both of interpolation and sterilization. I think it better, however, not to term the Floridean cystocarp a "generation," but detailed discussion of terminology would lead too far in this connection.

Why has the new Floridean plant body which arose so suddenly in their evolution no sex organs? It is probably because the diploid structure, for some reason, hinders this. On the other hand, immediately after the reduction division has occurred, a haploid generation arises which carries on the formation of gametes, etc. The sporophytic generation, which forms no gametes but spores only, is consequently a result of the delay of the reduction division.

From this it follows that this spore forming generation must be regarded as morphologically homologous with the original haploid one. This view approaches the so-called homologous theory of the alternation of generations of which I just now called PRINGSHEIM the founder.

It may be asked whether there are other groups of plants in which the same process may be conceived to have taken place. Yes! For example, the alternation of generations of *Dictyota* is capable of easy explanation in this way, and how, otherwise, can the perfect agreement of the gametophyte and the sporophyte be explained? The little we know of the diatoms seems to indicate that here, also, a similar delay of the reduction division has taken place. The plankton diatoms (*Diatomeae centricae*) are in all probability haploid, with the zygote as the only diploid phase, while the *Diatomeae pennatae* are diploid, the gametes being the only haploid cells. The second type has proceeded from the first by the delay of reduction division.

Might not this hypothesis also be made use of for the Archegoniates and phanerogams? The difficulties here encountered are considerably greater because the gametophyte generation has undergone such a tremendous reduction that hardly anything is left of it.

Meanwhile, it is noticeable that in recent times new views are being asserted respecting the relative degree of evolution of such plants, for example, as the liverworts. I am not a bryologist by profession, but I may here point out that GOEBEL (18) in the latest edition of his *Organography* expresses views concerning these plants which differ considerably from those which have been influenced by the so-called interpolation theory. GOEBEL's idea is that a comprehensive reduction has taken place in the series of the liverworts. Types like *Riccia*, with their nearly total devotion of the zygote to spore formation, are now placed not at the beginning but at the end of the evolutionary cycle, this condition being regarded as the result of extensive reduction. The much discussed *Anthoceros* also is regarded by GOEBEL as relatively primitive. It is noteworthy also that the well known stomata of the *Anthoceros* sporogonium are by no means to be regarded as new formations of the sporophyte.

They are indeed found fully developed, even on the gametophytes of other liverworts. Gametophytes and sporophytes, then, display much greater correspondence than was at an earlier date believed possible. On the homologous theory this is perfectly comprehensible. It is just the primitive forms which ought to display the greatest likeness in their two generations.

Another noteworthy feature found in the Pteridophytes is the fact that, in the Eusporangiates, for example, where the development of the sporangia is consummated inward from the original surface cells, the antheridial development on the prothallia likewise proceeds inward; while in the Leptosporangiates the development of both sporangia and antheridia proceeds from within outward. Still more examples of this kind might be quoted. What has been brought forward proves that, at any rate, far greater morphological correspondence prevails between gametophyte and sporophyte than had previously been assumed, and this seems to lend more support to the homologous theory of alternation than to the antithetical interpolation theory. I therefore wish to point out that the antithetical interpolation theory itself is not unassailable, but, on the contrary, must be regarded as being in important particulars no longer in full agreement with well known facts.

What is the bearing, in this respect, of the theory of migration? The fact that the larger groups of the higher plants represent as it were different stages in the migration of the plant world to land, of course, is too clearly manifest to be denied. The question may be raised, however, whether an exactly similar course of development, leading to the complete supplanting of the original gametophyte by an absolutely dominant sporophyte is not present also in organisms which have never migrated to land.

I believe that the Phaeophyceae present a very evident development of this kind. The noteworthy discovery by SAUVAGEAU (37, 38) of the alternation of generations in the Laminariaceae has thrown an entirely new light upon the whole life cycle of this group. We now know that the prominent phase of *Laminaria* is the sporophytic phase in an alternation of generations, while the gametophyte is a small, insignificant alga filament formerly perhaps described as a distinct species. Thus even here the sporophyte

generation prevails over the gametophyte to as high a degree as in the Pteridophyte group, although both generations still live in the sea.

Furthermore, this discovery regarding *Laminaria* is illuminating for algae of the *Fucus* type also. Few plants, I suppose, have been the object of such varied interpretation as *Fucus*. Algologists have long noted the anatomical similarities shown by *Fucus* and *Laminaria*. *Fucus*, however, is a sexual plant and *Laminaria* an asexual one. The discovery by STRASBURGER (41), FARMER and WILLIAMS (16), and YAMANOUCHI (54) that reduction division in *Fucus* precedes the formation of eggs and spermatozoids did not make the matter clearer. In accordance with what was known about ferns, it might formerly have been supposed that *Fucus* is haploid, but this was found not to be the case. The life cycle of *Fucus*, therefore, was often quoted with satisfaction by those who opposed the view that the alternation of generations is a nearly universal phenomenon in the plant world, and especially by those who denied the significance of the reduction division for the determination of the limits of the generations.

The discovery of the alternation of generations in the Laminariaceae, however, puts the life history of *Fucus* in another light. Several Laminariaceae, for example, *Saccorhiza* and *Laminaria*, especially in poorly nourished cultures, form gametophytes whose vegetative system is limited to one single cell which forms oogonia. Should this vegetative cell now be omitted in the development, so that the spores of the sporophytes fuse directly, and, moreover, should heterospory be added, then indeed the *Fucus* type is reached. The *Fucus* plant is, then, homologous with the *Laminaria* sporophyte, and also with the *Dictyota* sporophyte, but not with its gametophyte, as HARTMANN (21) and FRITSCH (17) incorrectly assume. *Fucus* "eggs" are therefore from one point of view to be regarded as spores (macrospores), while the spermatozoids are to be thought of as microspores, which, however, become gametes directly without the intermediation of a vegetative gametophyte stage. It should be noted in this connection that the Fucaceae are almost the only plants in the vegetable kingdom in which non-motile "eggs" are thrown out of their oogonia to be fertilized outside the mother plant. Everywhere else

non-motile eggs remain in their oogonia (or archegonia). But this behavior of these eggs is capable of easy explanation when we grant their homology with the spores of other Phaeophyceae. We find now that *Dictyota*, *Laminaria*, and *Fucus* form a continuous series of types from the viewpoint of the alternation of generations: *Dictyota* is an alga in which the generations are of equal size and evenly balanced; *Laminaria* one where the sporophyte completely dominates the insignificant gametophyte; and *Fucus* one in which the gametophyte generation has disappeared and the spores are the only haploid stage in the whole life cycle, and they have likewise become gametes directly. As regards the degree of reduction of the gametophyte, *Fucus* is at exactly the same point as the most advanced phanerogams. *Plumbagella*, examined by DAHLGREN (13), forms the most extreme type of development of the embryo sac of angiosperms, and in it the macrospore becomes directly, so to speak, the egg cell of the embryo sac.

The homology of *Fucus* has long been obscure and misinterpreted, chiefly I think because it is a sexual plant. Since the alternation of generations is often characterized as chiefly an alternation between a sexual and a sexless generation, *Fucus* was therefore viewed as the sexual individual in an alternation of generations. It is, however, not permissible to characterize this alternation solely with regard to sexuality, since to a certain degree sporophytes can also be sexual plants. This appears clearly from the heterospory, which is essentially a sex differentiation. I refer in this connection to the explanatory discussions of CHAMBERLAIN (8) and CORRENS (11). Hence the view held by certain authors, for instance COULTER (12), that it is wrong, from the strict standpoint of homology, to speak of "male" and "female" cones in the conifers, since sporophylls are organs in a supposed sexless sporophyte, is absurd. It is plainly this point of view which leads FRITSCH to call the conception of *Fucus* as a sporophyte "the height of absurdity." *Fucus* is a sexual plant of course, a "Gamont," to use HARTMANN'S (20) word, just as the vegetative phase of phanerogams is. This, however, does not prevent it from being, exactly as in the phanerogams, homologous with the spore-producing phase of a plant with a morphologically fully developed alternation of generations.

From this it is evident how misleading it is to characterize the alternation of generations as an alternation between sexual and sexless generations. On the contrary, it is a characteristic feature of this evolutionary process that in the course of the increasing disparity of the generations, in the direction of the dominance of the sporophyte, sexuality passes over more and more to the originally "sexless" generation. CORRENS (11) pointed out that it is more correct to speak of fern sporophytes as of "mixed sexuality" than as sexless.

Comparative studies have now shown that there occurs within the Phaeophyceae the same evolutionary trend with regard to the relation between gametophyte and sporophyte that we know in the series from mosses to ferns and seed plants; but in the case of these algae, this trend is not in any sense due to radically changed external conditions. There is, therefore, so far as I can see, no possibility of explaining the reduction of the gametophyte as being entirely due to the migration of plants to land, as is assumed by the migration theory.

What is the biological explanation of this rise and final predominance of the sporophyte over the gametophyte? I take it that this question cannot be answered except by considering the alternation of generations in connection with the reduction division. GOEBEL (18) and OLTMANNS (33) have asserted that it is a matter of indifference to plants when, where, and how reduction division occurs, but such a view seems to reveal an imperfect comprehension of the real nature of the reduction division.

After an account of the various places in which reduction division occurs in the evolution of the Thallophytes, GOEBEL (18) states:

It is thus no uniform picture which is afforded by the examples quoted. We see, figuratively speaking, that plants are concerned to bring about the event somewhere and in some manner, but they do not seem to care much at what point in evolution this occurs.

In particular, it has been from many quarters sharply emphasized that the cytological cardinal points, nuclear fusion and reduction division, and in general what has of late been called the alternation of nuclear phases, have no connection with the alternation of generations, characterized by HOFMEISTER on a morphological basis.

I do not share this opinion. Those investigators who consider that the position of reduction division in the life cycle of the plant is a matter of secondary importance, have laid too much stress on the rôle of the reduction division in "reconstructing" the double number of chromosomes; but the main significance of the reduction division surely lies rather in its rendering possible new combinations of chromosomes in the daughter nuclei.

In a certain measure reduction is to be regarded as the final act and the goal of fertilization. Very similar diploid nuclei at their respective reduction divisions can give rise to daughter nuclei differing very considerably, according as the chromosomes chance to combine. When the chromosome number of a plant is known, it can easily be calculated how many combinations are possible, and since at every reduction division, owing to the nature of the heterotypic division, two possibilities at most can be realized, it is easy to calculate the theoretically lowest number of reduction divisions required in order that all conceivable combinations may become possible. If the haploid chromosome number of a plant is a and its diploid consequently $2a$, then the number 2^a gives the number of possible different combinations of haploid nuclei, and the number 2^{a-1} is the theoretically lowest number of reduction divisions required in order that all possible combinations shall be able to be realized. These numbers are the same as those we know from the Mendelian hybrid laws for the different combination possibilities of pairs of characters. A plant with ten chromosomes in the haploid nucleus, and consequently twenty in the diploid, can by reduction divisions so combine them that $1024 (= 2^{10})$ different haploid nuclei can be formed, and for this at least $512 (= 2^{10-1})$ different reduction divisions are required.

Since, therefore, different diploid nuclei with exactly the same number of chromosomes are able to carry out reduction division with different results as regards chromosome combinations, it is clear that when a reduction division follows at once upon a fertilization (that is, one fertilization is only compensated for by one reduction division), never more than two chromosome combination possibilities can be realized; but when a more or less highly developed diploid sporophyte is formed, which produces numerous spore

mother cells, each of which undergoes reduction division (that is, when a fertilization is compensated for by many reduction divisions), numerous combination possibilities are sure to be realized. This is no unimportant matter when we are concerned with explaining the origin of the variety and the wealth of form found in the plant world. From this, moreover, it follows that it is not a matter of indifference when, where, and how the reduction division occurs in the life cycle of a plant. There are, broadly speaking, two distinct types of this in the vegetable kingdom. The fertilized diploid nucleus may either immediately undergo reduction division (thus displaying one fertilization, that is, one reduction division as in *Conjugatae Coleochaete*, haplobiontic red algae, etc.), or else the reduction division is postponed and a diploid sporophyte is formed, in which case several or many reduction divisions occur as a result of each fertilization, as in the diplobiontic red algae, mosses, ferns, phanerogams, etc. Not a single type in which reduction division is delayed and a diploid sporophyte or a diploid phase is formed (which compensates for fertilization by one single reduction division) is known in the whole of the vegetable or animal kingdoms, and in all likelihood such a type does not exist, although of course it is theoretically conceivable.

Proceeding from these considerations, the following working hypothesis may be postulated for the biological explanation of the origin of a sporophyte: the development of a diploid sporophyte, due to the delay of the reduction division, secures to the plant the possibility of bringing about numerous reduction divisions and thereby numerous character combinations. The rise of more abundant forms, and indirectly a larger number of fit types, is hereby rendered possible as the result of a fertilization. When, therefore, as in the *Coleochaete*, *Scinaia*, etc., reduction division follows immediately on fertilization, and only one haploid nucleus becomes further developed, all their spores are alike with regard to the assortment of chromosomes and to the inheritance combinations, although numerous propagating bodies, carpospores, are formed. On the other hand, in *Polysiphonia*, where reduction division is delayed and a diploid plant arises, various chromosome combinations can be realized at the different divisions of the many tetraspore mother cells.

The objection may now be raised that this delay of the reduction division is only of significance in hybrids, as a mere new combination of chromosomes would be meaningless unless something new were added. To this it may be replied that "hybridization" is only a special case, the crossing of species, of a universal blending of paternal and maternal properties occurring in all sexual organisms.

All sexually originated organisms are to a certain degree hybrids, if only the gametes are unlike, and in the majority of cases, except in complete homozygosis, they are unlike. Only when one and the same haploid plant forms male and female gametes which fuse with each other are they not unlike. In these circumstances most fertilizations are by gametes from different individuals, hence are to a certain degree hybridizations, and then certainly the delay of the reduction division is significant. One possible objection may be offered to this view, namely, that when in a haploid plant a great number of haploid gametes are formed in the gametangium, and these fuse with one another, this also results in many reduction divisions, and the plant has just as great a possibility of a diverse recombination of its chromosomes as if it formed a diploid generation with numerous spores arising by reduction division. To this the following reply can be given: a plant which is haploid can form in its gametangia only gametes of the same kind. The same diploid plant, however, thanks to the many reduction divisions, can form gametes of different kinds. In the former case, supposing that four different types of gametes (=factors) are present, there must also be four different types of haploid plants present if all the different combination possibilities are to be realized. Now four gamete types can be combined in sixteen different ways and nine genotypically different zygotes formed (cf. the general scheme of factors). Of these, four are homozygotes. In such individuals it is indifferent whether reduction division occurs immediately or is delayed, since they only form similar gametes. Eight zygotes are heterozygotic in one factor. Here at reduction division two different gamete types are formed, and on the supposition that at reduction division all the daughter cells of the tetrad are preserved, and not (as so often happens in nature) that three are destroyed, it is perhaps indifferent whether reduction division occurs at once or is postponed; but how

often does it happen that not all the daughter cells of the tretad survive, most of them degenerating. In such majority of cases a delay of reduction division is manifestly of advantage. Finally, four zygotes are heterozygotic in both factors. If reduction division occurs immediately, two types of gametes at most can be formed. On the other hand, if a diploid sporophyte is formed, followed by several reduction divisions, there is nothing to prevent the formation of all four gamete types as the result of fertilization.

Thus it follows that if reduction division occurs immediately, at least four fertilizations would be required instead of one in order to attain the same result, that is, to produce the four different possible types of gametes. Now, instead of four fertilizations, a single one can attain the same result. In the former case, not only four times as many gametes are necessary (eight instead of two), but also four times as many fusions. If the plant, by the delay of the reduction division, assumes a diploid structure, the originally haploid generation is reduced to the spores, and, finally, even these spores take the character of gametes; exactly as happens in the case of *Fucus*, this must be a far more advantageous organization than the haploid one. In this case (*Fucus*, phanerogams) sexuality has also migrated to the diploid sporophytes, and only the gametes are left as the last morphological remnant of the haploid generation. Thus it is more advantageous to the plant to become diploid than to remain haploid, since the diploid plant is not larger, demands no more room, assimilates as well, etc., as the haploid, and yet can, owing to its diploid structure, produce not only one but several kinds of gametes. What this means, when the ground is occupied by all sorts of plants among whom keen struggle for existence soon arises, may easily be understood. Assuming that for one species unlimited space was available, and further that possibilities of fusion could easily be realized, it would then perhaps be immaterial whether the plants developed as haploids or diploids. It is a fact, however, that everywhere in nature those organisms which can produce the greatest effect with the least expenditure of material gain the victory in the struggle. The diploid organism has done this, and that appears to me to be the reason why everywhere in the highest ranks of the vegetable kingdom the diploid generation predominates.

If this hypothesis be correct, then it must also be true that the first type (one fertilization—one reduction division) is a more primitive and more simple form of plant organization, and that the later type (one fertilization—many reduction divisions) comprises forms which, as is shown by other features, have reached a higher stage of evolution.

In the first type we may include Flagellates, plankton diatoms (*Diatomeae centricae*), Conjugatae, Chlorophyceae, the haplobiontic Florideae, and Phycomycetes. To the second type belong pinnate diatoms (*D. pennatae*), diplobiontic Florideae, Phaeophyceae, Myxomycetes, Ascomycetes, and Basidiomycetes, as well as Bryophytes, Pteridophytes, Gymnosperms, and Angiosperms.

A comparison between the red algae where reduction division is immediate, which the writer (45) has called haplobiontic forms, and the diplobiontic ones where several reduction divisions occur to each fertilization, shows unmistakably that the greater abundance of forms and species and the higher differentiation of the plant body are present in the latter. It is obvious, moreover, that the now-living Chlorophyceae and Conjugatae, which are almost all haploid, have not succeeded in raising themselves to equality with the more vigorous plant forms. On the other hand, we see in the Phaeophyceae a group that is incontestably the most highly organized and differentiated of all algae. I may mention the Sargassums, which, with their shoot differentiation and their leaf formation, etc., are little behind the phanerogams in shoot organization. The fact that the Phaeophyceae, as it is now proved, have, like the phanerogams, gone farthest among all the algae toward restricting most of their development to the diploid generation, cannot, it seems to me, be altogether an accident.

Fungi point the same way too. The haploid Phycomycetes display a far smaller variety of forms than the Eumycetes (Ascomycetes and Basidiomycetes). Properly speaking these can hardly be termed diploid, but the presence of the curious pairs of nuclei consorting in their cells generation after generation leads to precisely the same outcome, since many reduction divisions finally result from every nuclear fusion.

A comparison shows that wherever several reduction divisions

follow each fertilization, there are to be found more highly organized forms, and above all a noticeably greater variety of forms; that, briefly, this type must be regarded as being decidedly more highly evolved than the other, which is more primitive. The facts of nature also confirm the hypothesis that the origin of the alternation of generations, and therewith the origin of the diploid sporophytes, can be explained biologically as an organization advantageous to the plant for the attainment of numerous reduction divisions.

But how are we to explain the progressive reduction of the gametophyte? I believe it is owing to its having gradually become superfluous, since its function is only the production of many reproductive bodies, gametes, all genetically of equal value, with exactly the same stock of chromosomes; this, however, becomes superfluous when the formation of propagules is combined with the reduction division, and the haploid propagules formed thereby become gametes directly, as has been the actual course of evolution within the Phaeophyceae. The same thing appears from a study of the evolutionary cycle Pteridophytes-Gymnosperms-Angiosperms (final stage *Plumbagella*). The omission of the gametophyte stage denotes a high degree of simplification and abbreviation of the whole life cycle of the plant, without the number of sexual fusions being lessened thereby; instead, the number of reduction divisions has been increased, and these have been combined with the initiation of the propagation bodies.

Thus, from the point of view of the alternation of generations, it may be conceived that plants equipped with sexual reproduction have developed, broadly speaking, from haploid single organisms, haplobionts (Chlorophyceae of the *Spirogyra* type), with only a cytological alternation of generations or a nuclear phase alternation, to haploid and diploid double organisms, diplobionts (*Dictyota*-type, Pteridophytes), with both nuclear phase alternation and morphological alternation of generations, and thence, finally, on to the now diploid haplobionts (*Fucus*, phanerogams), likewise with almost entirely nuclear phase alternation but without a manifest morphological alternation of generations, this having finally disappeared.

On the whole, the history of development shows that plants, after having been originally haploid, have passed on to become dip-

loid. The purely morphological alternation of generations is in appearance a kind of transition stage, lacking at the outset, and at the end once more disappearing. This hypothesis has the advantage of being a general explanation of the rise of the alternation of generations in all groups, independently of their external conditions of life.

This hypothesis is offered as an explanation of the alternation of generations in the vegetable kingdom. I am very conscious, of course, that, like all other hypotheses, it does not answer all the questions which may be raised; but the value of a hypothesis depends, so to speak, upon how many different questions it can bring under a single general point of view. I believe I have shown that if the alternation of generations is recognized as a characteristic of practically all plant groups which have fertilization, and if this view is compared with the new facts which modern genetic research has discovered, we shall get nearer the truth than by regarding the alternation of generations as one of the changes of organization which occurred with the migration of the plant world to land.

Every hypothesis has a limited value, and does not contain the whole truth. On the other hand, it must not be forgotten that every hypothesis which once could claim to be reasonable always preserves some truth in itself, even though in due time it has to yield to a new and better explanation.

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NATURE OF THE MULTIPLE SEEDED XANTHIUM
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 369

CHARLES A. SHULL

(WITH NINE FIGURES)

Occurrence

Multiple seeded specimens of *Xanthium* occur rarely in nature, but it has been the writer's good fortune to obtain such material from three different localities during the last fifteen years. The occurrence of these three single specimens is so far apart in time and space as to preclude any genetic connection between them. The first burs of this character ever reported, so far as the writer is aware, were those obtained by Mr. F. F. CREVECOEUR near Onaga, Kansas, in the summer of 1909. The name used by CREVECOEUR for this material was *X. canadense* var. *globuliforme*. He adopted this designation because he believed it was derived from *X. canadense*, on the ground that the second generation produced some plants with simple burs of the *canadense* type. Since this date the genus *Xanthium* has been monographed by MILLSPAUGH and SHERFF (7), who go back to the name *chinense* for this species, and by WIDDER (11), who makes *X. canadense* a synonym of *X. pungens*. If this interesting form is to bear only a varietal name, it would be known as *X. chinense* var. *globuliforme*,¹ or *X. pungens* var. *globuliforme*, according to which synonym we choose for *X. canadense*.

This material ran through three generations, counting as the first generation the original parent, which was found growing in a corn field. There was a strong tendency toward sterility, but the second generation produced some seeds in Mr. CREVECOEUR's flower garden. The third generation, grown by Miss MEEKER at Ottawa, Kansas, in 1911, was entirely sterile. A few burs of the earlier generations came into my hands in 1914, but the seeds were no longer viable, owing to the death of the hypocotyls. Many of the cells of

¹ The name *X. canadense globuliforme* Crevecoeur has been used for this form by MILLSPAUGH and SHERFF, North American Flora 33: 39. 1922.

the cotyledons were still alive, but no germinations were obtained. A photograph and drawings of these burs were published (9), with some suggestions as to the possible origin and significance of the many seeded burs.

It was not until thirteen years after the first appearance of the type at Onaga that a similar plant was found in nature. In the autumn of 1922, Mr. A. A. HANSEN found a multiple seeded specimen near Richland, in the southeastern part of Rush County, Indiana. This is at least 500 miles from Onaga. The burs were sent to the Field Museum, and reached me through the kindness of Dr. E. E. SHERFF, to whom they had been referred for identification. The seeds were tested and found to be viable. Sixteen seeds were found, and from these sixteen plants were grown during the summer of 1923 in the garden of the Hull Botanical Laboratory. A brief note was published (10) at the time, but no detailed account of the material has been given. The plants were very late in blooming, although the season was of normal length. This late blooming was accompanied by what seemed at the time abnormally vigorous vegetative activity. The stems were of enormous size for cockleburs, fully double the diameter of ordinary individuals, and with lateral branches many feet in length. The only photographs taken of this material were of the full grown plant, which is shown in fig. 1, and the fasciated stems, fig. 7. The general impression given to the observer was that they were a *gigas* type of plant, so far as structure and vigor indicated.

The male inflorescences were only in bud in the last days of August and early September, and the female flowers were quite small. Many of the burs were still immature when severe frosts cut the plants off. Attempts were made to remove some of them to the greenhouse to complete the development, but these were not successful. The plants lost their leaves and died. The crop of burs was therefore small, and most of them prematurely ripened. Examination of hundreds of burs, the largest and best that could be selected, showed almost complete sterility. The involucres were much more deeply cleft than those of the original parent plant, and fewer floral cavities developed.

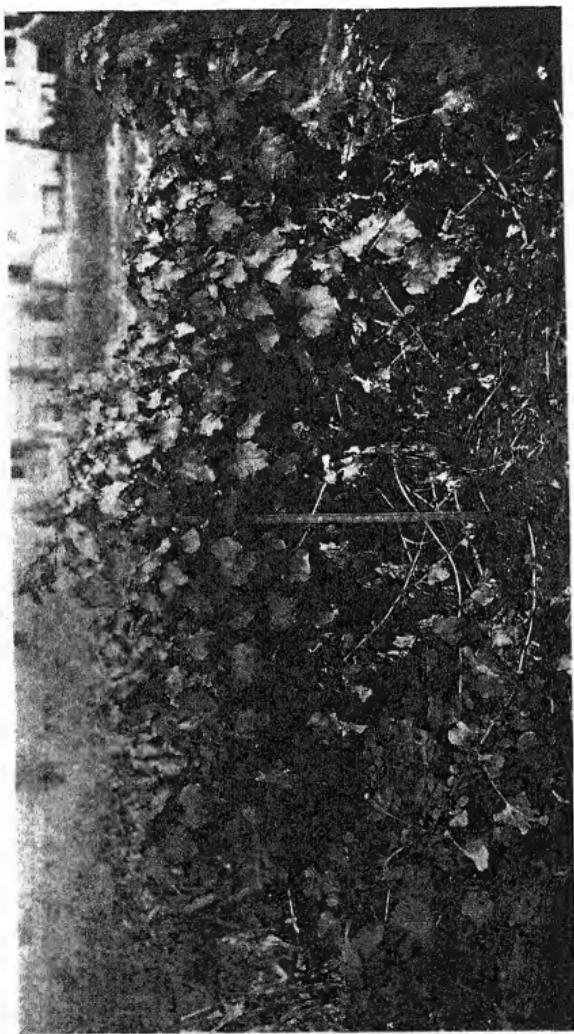


Fig. 1.—Mature plant, from second generation of the Indiana material; note enormous vigor and excessive branching.

Some months later the material was examined by Dr. R. O. EARL, who found after prolonged search a few small, shriveled seeds. From these the third generation was grown late in the summer of 1924. Although the plants were grown in the greenhouse, they began to bloom at about six weeks from the seed, at the same season of the year as the previous generation in the field. The plants ripened off



FIG. 2.—Original stem of Kansas City specimen, 1925

soon after flowering, and none of the burs contained seeds. Attempts to prolong the vegetative life by rooting cuttings were unsuccessful, although the cocklebur can be propagated in this way during the early part of the growing season. The cuttings of this late summer generation, however, continued to ripen their tissues, and died without entering into a vegetative growth period. A higher nitrogen supply might have made a difference in the growth behavior, es-

pecially if it had been accompanied by a longer day, which is known to favor vegetative activity. Thus the material of this second discovery perished without establishing a strain of many seeded burs.

Fortunately a third specimen of the same type was secured in 1925. This specimen was found in the latter part of September by Mr. O. C. DURHAM, at the corner of 70th and Indiana, Kansas City, Missouri. The plant was still green, but the burs were already brown. The specimen was preserved with the burs on the stem. The general appearance of this parent stalk with the original burs is shown in fig. 2. I am greatly indebted to Mr. DURHAM for the specimen, and for having the photograph made. As the second generation has produced an abundance of seeds for a third generation, an account of the habits as observed during the last few years will be given.

Observations on growth

On opening some of the burs from the specimens of fig. 2, well developed seeds were found. There are usually about six good seeds in each bur, but as many as eleven have been found. The seeds are usually found in the outer row of ovarian cavities, but on several occasions good seeds have been obtained from interior cavities. In one of these burs with ovarian cavities for about sixteen seeds, the six good seeds shown about natural size in fig. 3 were found. All were from the outer circle of florets. The seeds are smaller and narrower than those of the common species, and have a different form. They are triangular in cross-section, with one side of the triangle tangent to the outer wall of the involucre. This form is the result of compression of the developing seeds. The seeds are not distinguishable by shape and size as uppers and lowers, nor by position in the burs. Germination of 100 per cent of the seeds on several occasions indicates little tendency to delayed germination, but Dr. E. L. REED reported one seed that did not germinate until its coat had been broken.



FIG. 3.—Six seeds from single bur of plant in fig. 2.

The six seedlings developed from the six seeds in fig. 3 are shown at an early stage in fig. 4. The photograph was taken May 10, 1926.

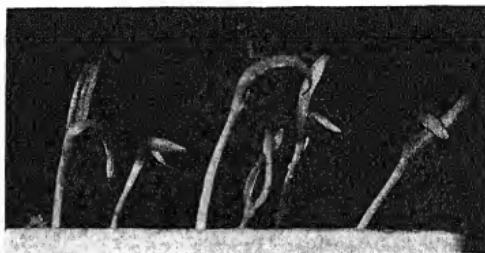


FIG. 4.—Seedlings of multiple-seeded *Xanthium*



FIG. 5.—Specimen of multiple seeded *Xanthium* grown at Stephenville, Texas, July 14, 1926.

In order to insure a longer season of development, some of the seeds were sent to the John Tarleton Agricultural College, Stephenville, Texas. Through the kindness of Dr. REED and his gardener two plants were raised to maturity. Several other plants were accidental-

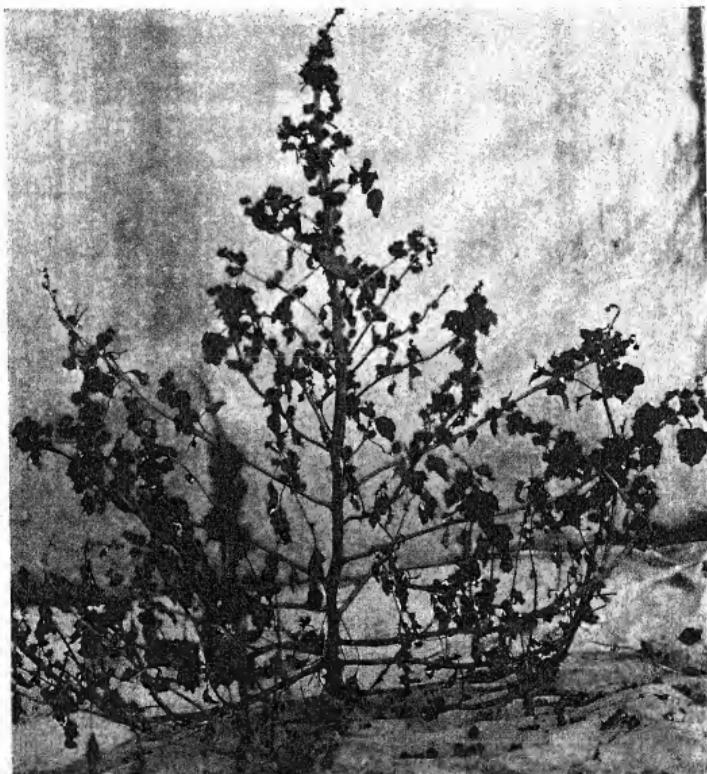


FIG. 6.—Mature plant with ripe burs, Stephenville, Texas

ly destroyed through weeding operations. The plants grew vigorously, as is shown by the photograph, fig. 5, which was taken on July 14, 1926. The precaution of sending this material to Texas was found to be unnecessary, for it proved to have a much earlier blooming time than the offspring of the Indiana material of 1923. The

twenty-five specimens grown in the greenhouse and garden at Chicago also bloomed early enough to ripen thoroughly before frosts.

A full-grown mature plant with ripe burs, at the close of the season, is shown in fig. 6. The general habits of growth are beautifully shown in the photograph. The plants are large and vigorous, with very long lower branches, and numerous branches per plant when

grown in the open. In the greenhouse and garden at Chicago all the plants were grown in pots, so that removal to the hothouses would be possible in case of late flowering. The soil mass was not sufficient for maximum growth, and these specimens were by no means so large as the one shown in the figure, but all produced good plants.

Fasciation

A remarkable observation was made in connection with the material sent from Indiana by Mr. HANSEN. Of the sixteen plants grown to maturity, about 75 per cent of them showed a pronounced flattening of the stem, and those that

FIG. 7.—Fasciated stems of multiple seeded *Xanthium*, from Indiana material in second generation.

did not show fasciation were excessively branched. This may properly be considered the morphological equivalent of fasciation. Parts of several stems that showed strong fasciation are shown in fig. 7. Examination of the stems failed to disclose injuries which might have caused this. The condition of the parent plant is not known, but in the third generation the fasciation was apparently inherited by all of the specimens. These were grown in the greenhouse where insect injuries were never observed.



The 1926 offspring of the specimen found in Kansas City in 1925 have not shown as pronounced fasciation as the Indiana material. However, if one examines the tip of the stem in fig. 2, it is found to be distinctly flattened, although the lower parts are more nearly terete. In the absence of the writer from Chicago in the late summer and autumn of 1926, the twenty-five specimens grown in the greenhouse and garden were examined by Professor C. J. CHAMBERLAIN, who expressed the opinion that practically all of the specimens showed some signs of being fasciated. In some cases the plants were broadly flattened. It seems, therefore, that fasciation is prominently associated with the multiple seeded condition. The possible significance of this will be considered in the discussion of the causes of the many seeded type.

Photoperiodism

In common with various species of *Xanthium*, the multiple seeded form exhibits a certain amount of responsiveness to photoperiods. Some years ago a number of species were grown at the University of Kansas, and it was observed that each species tended to have its own blooming time. This behavior was spoken of as physiological isolation (8). The interesting studies of GARNER and ALLARD on photoperiodism were not available at that time. It is clear now that the *Xanthium* species have the same reason for different blooming periods as, for instance, soy beans, which were found to be strongly photoperiodic.

When the multiple seeded strain from Indiana began blossoming very late in the summer (September), it was thought to be a photoperiodic peculiarity. The material was believed to have the shortest photoperiod of any of the species of *Xanthium* which had been grown. The generation raised by EARL, in the summer of 1924, blossomed at the same time in September as those which were planted in May, 1923, which indicates an inherited tendency. However, there is evidence that material of other origin may have a very different blooming response. The Kansas City material sent to Texas had distinctly visible male inflorescences on July 8, 1926, nearly two months earlier than the Indiana specimens; those raised in the garden and greenhouses at Chicago also showed a much earlier

blooming period. Each strain of the multiple seeded form appears from this to have its own individual blooming behavior.

That the control of blooming in *Xanthium* is not exercised by photoperiod alone was first observed a number of years ago. Seedlings started in the hothouse (25° - 27° C.) developed an apical cluster of male flowers in about two weeks from the seed; but when grown in a colder house (10° C. at night) the plants came to blooming in about four months. As the photoperiod was the same for both at the start, it was evident that temperature greatly modified the blooming response. GILBERT (3) has since made a study of this behavior, comparing the metabolic conditions of short-day high-temperature and long-day low-temperature plants. He found a rising carbohydrate-nitrogen ratio as the plants approached the blooming period; but the value of the ratio was lower in the plants that bloomed very quickly from high temperature than in those that blossomed only after a long growth period at lower temperatures.

If there is such a thing as a vegetative-reproductive nutritive equilibrium in plants, it seems to be reversible in *Xanthium*; for if a plant which has been induced to blossom in short-day high-temperature conditions is removed to long-day low-temperature conditions before the maturity of tissues has progressed too far, the plant will become once more vegetatively active by developing new lateral branches. The blossoms already formed may produce seed, but the new axillary branches grow vegetatively a couple of months and come to blossoming at the usual time. In this way we have succeeded in producing two periods of blooming, months apart, in a strictly annual plant.

The multiple seeded forms will probably show the same behavior in every detail. Seeds set to germinate March 11, and planted in pots in the warm greenhouse (25° C.) on March 14, 1927, had visible terminal male flower clusters in nine days from the time of planting. When kept in a box to control the day length to seven hours, the development of the precocious inflorescence was a little more pronounced than when the natural day length was given.

Five of the multiple seeded plants are shown in fig. 8. The smallest seedling, no. 1, was seventeen days old at the time it was photographed. It had been kept in the cold greenhouse, regulated to

10° C. at night. The two plants next to the right are the same age, but grown in the hothouse. No. 2 was grown with a 7-hour day, while no. 3 had the full length day of early April. The two plants at

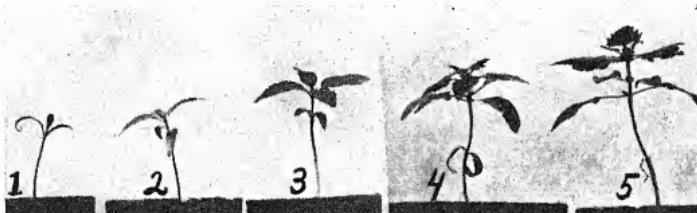


FIG. 8.—Temperature response: 1, in cool house 17 days; 2, warm house 7-hour day; 3, warm house full day; 4, warm house 7-hour day, 36 days old; 5 warm house full day; plants 2 and 3 are in bud, and 4 and 5 ready to shed pollen.

the right were thirty-six days old, no. 5 having had the full length day and no. 4 a 7-hour day. These plants had just one cluster of male flowers, the terminal cluster, and the female flowers occupy all of the leaf axils except those of the cotyledons. In fig. 9 is shown the leaf display of the 7-hour plant no. 4.

Plant no. 5 began to shed pollen on April 12, at thirty-two days, about twenty-three days after the buds first became visible. Plant no. 4 shed its first pollen on April 18. The short day apparently retards development at this particular season. It is seen also that the full-day length plants have longer internodes than the short-day plants.

It is remarkable that plant no. 4, almost ready to bloom, still has its cotyledons in active condition. The cotyledons on plants in full-day length shriveled much sooner than those in the 7-hour day.

As the day length increases, the tendency to precocious blooming decreases, and finally ceases altogether. This behavior is identical with that observed by GILBERT in the common species of *Xanthium*.



FIG. 9.—Vertical view of plant no. 4, fig. 8, showing leaf development and terminal male inflorescence; plant 33 days old.

thium. During the longer day season, flowering can be induced by shortening the length of the illumination period, in plants kept at high temperatures. Temperature and photoperiod seem to be about equally important in determining the behavior of the organism.

Possible causes of multiple seeded condition

Originally the writer was inclined to believe that the multiple seeded bur was the result of a reverisional mutation toward remote ancestry; that the many seeded capitulum represents the type of inflorescence in the progenitors of the cockleburs of this age. From this many seeded bur our two seeded burs have come by a process of reduction, if we accept FARR'S (2) interpretation of the inflorescences of *Xanthium*. Such reversions to ancestral floral conditions have been noted in other species of Compositae, as by COLLINS (1) in the case of hybrid forms of *Crepis*. This interpretation should be kept in mind, for it is a logical explanation of the phenomena.

There has always been a question as to whether hybridization might be a factor in the production of this type of plant. Although CREVECOEUR's observations of the multiple seeded species of *Xanthium* in 1910 indicated a hybrid condition (9), such splitting into groups of many seeded and 2-seeded forms has never been observed in any of my cultures. The inheritance of the multiple seeded condition has been perfect in every generation thus far grown. Self sterility in the first two strains might be thought to indicate hybrid condition. JEFFREY (4, 5) has championed the idea that self sterility is a mark of hybridization, but it is conceivable that other causes might be responsible for it, as for instance the fasciated condition observed in these forms.

Indeed, one is led to inquire whether it may not be possible that the multiple seeded condition is merely the expression of fasciation, carried out into the burs. Is one of these burs simply an aggregation of a dozen or so burs into one, a fasciated bur? Investigations have not gone far enough along this line to answer this question, but the way seems open now to test certain phases of the matter experimentally. Miss JOHNSON (6) has recently shown that *Helianthus annuus* can be caused to fasciate its stems, leaves, and flowers by treatment with the X-ray. Since some other Compositae have also

shown a tendency to fasciate after exposure to X-rays, Miss JOHNSON has undertaken to determine whether the ordinary species of *Xanthium* with two seeds to the bur can be caused to fasciate in this way, and whether such induced fasciation leads to the production of multiple seeded female inflorescences.

While it is possible, therefore, that we have here a mutational reversion to a far distant ancestral condition of inflorescence, the fact of the association of fasciation with the multiple seeded condition of the bur should lead to caution in the interpretation. How far can we go in linking sterility with fasciation is a question. At present we know little about the cytological details of pollen formation and the development of the female gametophyte in the ordinary species of *Xanthium*. Chromosome behavior during cell division in normal and fasciated meristems has never been studied. Fasciation may not be accompanied by any spindle irregularities, or chromosome aberrations, but it is possible that it is. The morphological and cytological details of the meristems of these different types of growth forms should be known before we attempt to go any further with the interpretation. The chromosome numbers of the normal and multiple seeded forms should be known, to determine whether the vigorous growth of the many seeded type is at all associated with a doubling of the chromosome number, as in the *gigas* *Oenotheras*. No such doubling is expected in this case, but the facts should all be known. The last specimen obtained from Kansas City has provided a great supply of material suitable for such morphological and cytological studies.

Summary

1. Multiple seeded specimens of *Xanthium* have been found in nature on three occasions, in 1909, 1922, and 1925.
2. The first two groups, after being grown for two generations following the original plants, perished because of infertility.
3. Plants grown in 1923 showed very marked fasciation, which was exhibited by 100 per cent of the two generations. It is believed to be strictly inherited. The 1925 material showed less pronounced fasciation in the generation grown in 1926, but nearly all specimens showed evidence of this modification.

4. It is suggested that the fasciation may have something to do with the partial sterility of this variety of *Xanthium*.

5. The multiple seeded condition may have arisen as a reversion toward remote ancestral floral conditions, or it may be associated with the fasciation. In the former case, the bur would represent a many-flowered capitulum from which our present two-flowered capitulum has been derived by reduction. In the latter case, the bur would represent a dozen or more burs consolidated into one through fasciation.

6. This form of *Xanthium* exhibits the same peculiar photoperiodic and temperature responses as the common species of the Chicago region. The vegetative-reproductive equilibrium seems to be reversible, as an induced blooming period may be followed by vegetative activity and a later blooming period of the vegetatively formed branches.

7. The multiple seeded variety of *Xanthium* presents some unique and interesting problems of anatomy, cytology, and floral development.

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ANATOMY AND DEVELOPMENT OF TOMATO FLOWER¹

DELMER C. COOPER

(WITH PLATES XI, XII AND SEVEN FIGURES)

The literature of recent years concerning the tomato, *Lycopersicum esculentum* Mill., contains the reports of many investigations in the fields of pathology and physiology, and comparatively little about the morphology of the plant. Study of the morphology and anatomy has been secondary to that of nutrition and disease.

For the investigation of the anatomy and morphology of the flower here reported, two well known varieties were used, Bonny Best and Greater Baltimore, since these could be obtained in abundance in the greenhouses of the Purdue Agricultural Experiment Station.

Gross morphology

The flower cluster of the tomato is a short, forked racemose cyme of seven to twelve flowers (fig. 1). It is unusual for more than two flowers to open at one time in each inflorescence, so that a peduncle is often found bearing small fruits, open flowers, and developing buds at the same time (fig. 2). The tomato flower is perfect, hypogynous, regular, pendant, and typically six-merous (fig. 3). The calyx tube is very short, and bears six leafy lobes which are linear to lanceolate (fig. 4). The calyx is persistent and increases in size with the development of the fruit. The rotate corolla consists of a short supporting tube and six broad lobes. It is a pale greenish yellow when the flower is partly opened, and the thin lobes are much wrinkled. In the fully expanded flower the lobes are much reflexed and a brilliant yellow.

The six or more orange-yellow stamens (fig. 21) are attached adnately to the throat of the corolla tube, and are connivent or syn-

¹ Contribution from the Biological Laboratories, Purdue University, Lafayette, Indiana. A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science.

egesious, that is, they are laterally coalesced to form a hollow cone around the pistil, which occupies the central portion of the flower.

The inconspicuous pistil is pale yellowish green, and consists of the enlarged basal portion or ovary, the elongated style, and the rather flattened stigma which extends slightly beyond the apex of the androecium (fig. 18). The pistil is composed of six fused carpels

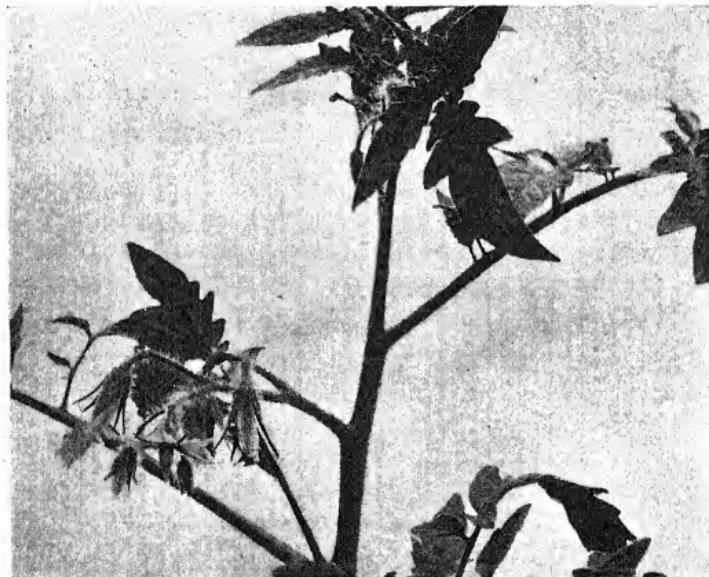


FIG. 1.—Flower cluster showing pendant nature of flowers; natural size

and the ovary is typically six-celled. The corolla and androecium fall off soon after pollination, and the style follows not long afterward.

It is of interest to note that BAILEY (1), GRAY (4), and PAYER (7) describe as five-merous the whole order Solanaceae. THOMPSON (9) reports the same condition in his description of the tomato itself, and BRITTON and BROWN (2) consider the flower as being five-merous, rarely six-merous. On the other hand, many workers, not especially concerned with the morphology of the flower, have recorded the six-merous condition.

Examination of more than 1000 flowers showed that the varieties studied are basically six-merous. The first 200 flowers revealed two cases of numerical variation due to a multiplication of parts. Only one of the blossoms out of the total number examined was found to be five-merous throughout. During the summer of 1926 the flowers on tomato plants in many gardens were examined, and were found to

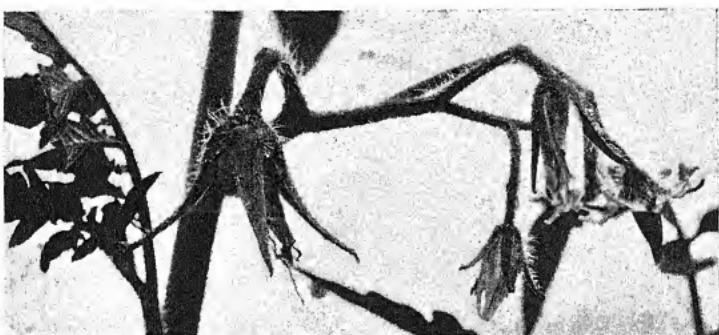


FIG. 2.—Peduncle bearing very young fruit, mature flowers, and unopened flower bud; abscission region can be seen on pedicels of several flowers; natural size.

be typically six-merous. It was discovered, however, that the flowers of the pear-shaped tomato, *L. pyriforme*, are five-merous.

Development of floral organs

For the microscopic study, developing flower buds in various stages of growth were gathered from healthy plants, fixed in a solution made by adding 6.5 cc. of formaldehyde and 2.5 cc. of glacial acetic acid to 100 cc. of 50 per cent alcohol, dehydrated, cleared in chloroform, and imbedded in paraffin. Both cross and longitudinal sections were cut, 12 μ in thickness, mounted serially on slides, and stained in safranin and Delafield's haematoxylin. Some small buds and parts were similarly fixed, stained, dehydrated, cleared in xylol, and mounted *in toto* for a study of the gross anatomy.

In the development of the flower, a small protuberance of meristematic tissue grows out from the pedicel of the preceding flower. The portion of this pedicel posterior to the protuberance becomes part of the peduncle. In the case of the first flower of the cluster,

this meristematic protuberance or axis grows from the axil of the leaf. The first primordium of the floral organs develops on one side of the meristematic protuberance when the latter is of microscopic size. Other primordia develop, one after another, passing around the protuberance in a clockwise manner until a ring of six (fig. 19) is formed on the circumference. By the time that this cycle is completed the first primordium has developed to some extent, and the others

in proportion, so that each is somewhat longer than the one subsequently produced. Growth of each of the first six rudiments is more rapid on its dorsal side, so that concave structures are produced which arch over the apex of the axis (fig. 19). Thus the calyx forms a protective covering over the growing tip of the protuberance, the apex of which becomes the receptacle.

The primordia of the second whorl grow out in the same manner, except that they are slightly higher on the axis and are alternate with the calyx

primordia. The second whorl develops into the corolla. Next appears the third or staminal whorl, the primordia of which are opposite those of the first whorl and alternate with those of the second. Similarly, the primordia of the fourth whorl, which develops into the pistil, are alternate with those of the preceding one (figs. 20, 21). The floral organs are thus developed in a low spiral arrangement of four turns around the receptacle, the fourth whorl forming the apex.

The development of the primordia of the outer circle is such that they broaden at their bases until they touch one another laterally, after which the entire ring of meristematic tissue grows upward, forming a low cylindrical calyx cup. The calyx, therefore, is not

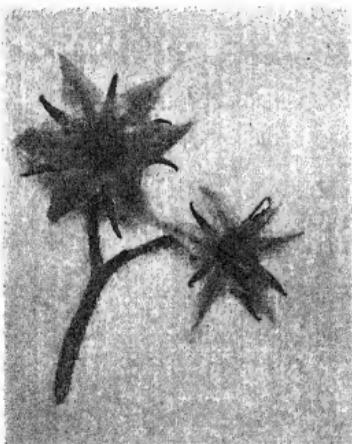


FIG. 3.—Tomato flower showing six-merous condition; natural size.

formed of sepals which were originally free and later united laterally at their bases, but rather is formed by the outgrowth or protrusion of a ring of meristematic tissue basal to the early whorl of six distinct and separate primordia.

Protected by the developing calyx, the second whorl of primordia, the forerunners of the corolla lobes, develops slowly in comparison with the other whorls of the flower bud. The formation of the corolla tube is similar to that of the calyx tube. Just before maturity of the bud the corolla makes rapid growth, and finally pushes the calyx open. As the corolla tube grows out from the receptacle, the six meristematic regions of the third or staminate whorl of primordia grow up with it in such a way that the stamens are adnate to the interior of the corolla tube, and appear to have developed from it.

Each primordium of the staminate whorl or androecium consists of homogeneous tissue, which, as growth continues, becomes differentiated into distinct parts. The apical portion of each stamen becomes the anther, and consists of four lobes of sterile tissue and sporeogenous cells. The basal portion develops into an elongated filament adnate to the corolla tube, except for the rather thick free stalk which terminates in the anther.

The fourth whorl of six primordia develops into a compound pistil consisting of a group of six united carpels. The placenta on the inner or axillary wall of each carpel enlarges, so that it, with the developing ovules, nearly fills the locule (fig. 16). The development of the ovary is uniform from the first.



FIG. 4.—Young inflorescence showing hairy condition and position of flower cluster at apex of plant; X10.

Anatomy of mature floral organs

PEDICEL.—The pedicel which supports a single flower, as well as the peduncle from which it arises, is composed of a rather thick cortex, a ring of vascular tissue, and a central portion of pith (fig. 22). The epidermis of the pedicel is composed of long narrow cells covered with a cuticle, and contains relatively few stomata. These are arranged in such a way that the stomatal openings are parallel to the long axis of the pedicel (fig. 9). Long multicellular non-glandular hairs, and short stalked glandular hairs tipped with four cells are scattered over the surface of the pedicel (figs. 11, 12, 13). The ab-

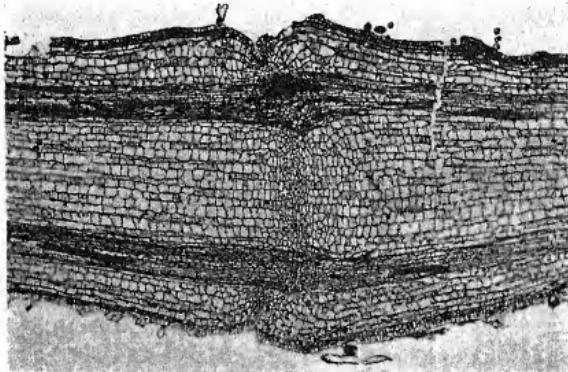


FIG. 5.—Photomicrograph of longitudinal section of pedicel showing abscission layer; $\times 40$.

scission layer (fig. 5) of the flower is formed about midway in the pedicel, or about 1 cm. below the receptacle. At this point the pedicel is somewhat constricted, and the individual cells are small and much flattened. These small cells later become more or less corky, and finally separate with the ripening of the fruit, so that the pedicel breaks at this point.

The receptacle becomes a rather large, very flat disk, within which the vascular cylinder divides into strands which lead to the various organs of the flower (fig. 14). These strands or bundles radiate outward, one to each of the floral parts; consequently bundles leading to the corolla lobes are opposite the sinuses of the calyx.

CALYX.—The first six vascular bundles leading from the vascular cylinder in the receptacle radiate outward, one to each lobe of the calyx (fig. 24). Each bundle becomes a finely branched and anastomosing system of veins leading to all parts of the lobe. The cellular structure of the calyx lobes is similar to that of the leaf. Each lobe is about fifteen cell layers in thickness through the midrib. The mesophyll is rather spongy, and its cells contain many chloroplasts. The epidermis is composed of a single layer of small cells covered with a thin cuticle.

Stomata are found in great abundance on the dorsal surface of the calyx, especially toward the base of each lobe and on the calyx tube. These stomata protrude above the surface of the epidermis (figs. 8, 15). Those on the ventral surface of the calyx are small, and are not raised above the general surface. An intercellular space or substomatal cavity is formed under each stoma.

Several types of hairs are found on the surface of the calyx. Those on the dorsal (lower) surface vary from short unicellular hairs to long multicellular hairs (figs. 11, 12). Many of the longer hairs are supported by a group of cells which protrude above the general surface of the calyx. The glandular hairs (fig. 13) are similar to those occurring on the pedicel, except that the stalks of the former are longer. The single celled hairs are simply an outward extension of individual epidermal cells. Papillae and short stalked hairs are found on the ventral surface of the calyx lobes. The calyx, unlike the corolla and androecium, which disappear soon after pollination, is persistent and continues to grow.

COROLLA.—The second zone of six vascular bundles originates in the vascular cylinder of the receptacle just above the first zone, and enters the corolla, a single bundle passing into each lobe (figs. 25, 26). The epidermis of the corolla is composed of a layer of thin walled cells, some of which on the ventral surface, especially at the apex of the lobes, protrude out into papillae (fig. 10). Many glandular hairs with very short stalks are present on the dorsal surface. No stomata were observed on the corolla. The lobes of the corolla are about eight cell layers in thickness, and consist largely of spongy mesophyll. The color is due to yellow chromoplasts within the cells of the mesophyll.

ANDROECIUM.—The third zone of six vascular bundles originates in the vascular cylinder of the receptacle, just above and alternating with those of the second whorl (figs. 27, 28). The stamens are carried upward with the growth of the corolla tube. The short free portions of the filaments thus seem to have developed from the throat of the corolla, rather than from the receptacle; however, the vascular trace of each stamen passes as an unbranched bundle from the receptacle through the adnate filament to the anther. Each anther is divided longitudinally into a right and left lobe, separated by the connective tissue. Each lobe contains two microsporangia which extend approximately the full length of the anther. At maturity the dorsal side of the anther is covered with large papillae. The ventral side has a few hairs, especially at the apex. There are no hairs along the line of dehiscence. With the exception of the pollen grains and the vascular bundle, each stamen consists of parenchyma cells surrounded by an endothecium. Stomata apparently are lacking on all parts of the stamen.

Although the flower (figs. 20, 21) is typically six-merous throughout, individual flowers occur which possess a variable number of stamens. In cross-section it was occasionally found that one or more filaments might contain two vascular bundles. Such filaments were surmounted by two anthers. The two vascular bundles of such filaments, however, are found to result from a branching of one of the six bundles arising from the stele of the receptacle (figs. 28-31).

Four microsporangia, two to each lobe, are formed in the anther, and extend approximately its full length. In cross-section each microsporangium appears somewhat horseshoe-shaped (fig. 6). After the development of spore mother cells from the sporogenous cells, the conjunctive or sterile tissue separating the sporangia of each lobe breaks down, leaving one spore chamber (pollen sac) in each lobe (fig. 7). Later each spore mother cell divides into four cells, each of which is a microspore. The microspores develop thickened cell walls and become pollen grains.

When the corolla begins to open, the stamens are pale yellow, and the spore chamber is closed. After a period of 24-48 hours, depending on atmospheric conditions, the color of the stamens changes to a bright yellow and dehiscence begins. This is accomplished by a long-

tudinal splitting of the spore chamber wall along its weakest part, which is the place where the conjunctive tissue has recently broken down. This splitting is introrse, so that the pollen grains fall on the stigmatic surface of the pistil.

PISTIL.—The vascular bundle extending into each carpel divides, so that one branch passes outward and upward in the pericarp, and

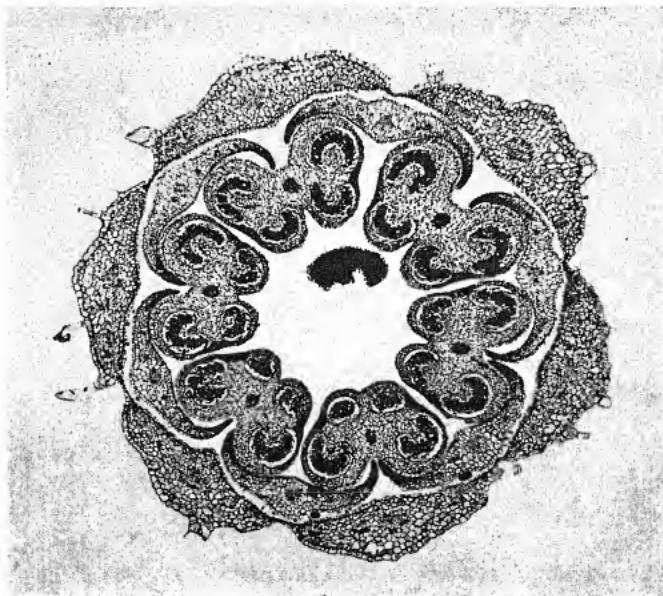


FIG. 6.—Photomicrograph showing six-merous condition of flower bud as seen in cross-section; $\times 50$.

continues up through the style to the stigma (figs. 28, 30), while the other passes through the placenta and divides into many smaller bundles, each one ending in an ovule (fig. 31). The papillae of the stigmatic surface secrete a sticky fluid in preparation for pollination.

Small papillae similar to those on the corolla (fig. 10) are scattered over the epidermis of the ovary, and extend well up on the style. Large papillae and multicellular glandular and non-glandular hairs are found on the basal half of the style and on the ovary.

Rather large stomata are sparsely scattered over the style, but none could be found on the ovary. MAKEMSON (6) reported stomata and lenticels on the ovary, but GROTH (5), ROSENBAUM and SANDO (8),

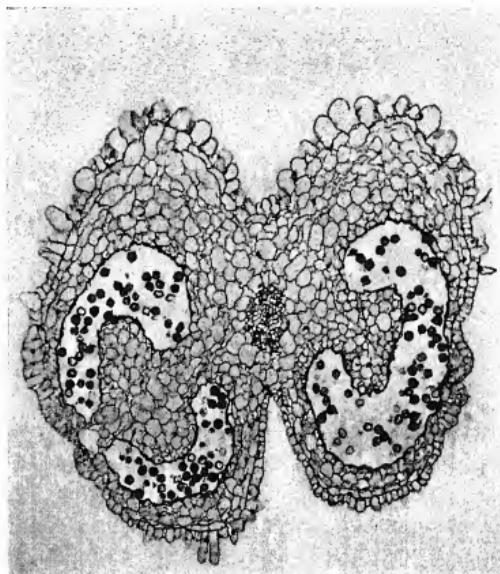


FIG. 7.—Photomicrograph of cross-section of anther showing mature pollen grains and disappearance of conjunctive tissue; $\times 85$.

and GARDNER (3) found no stomata on the ovary, although the last named, like the writer, noted their presence on the style, receptacle, calyx, and pedicel.

Summary

1. The inflorescence of the varieties of tomatoes studied is a racemose cyme bearing 7-12 flowers.
2. The floral organs are developed in a low spiral arrangement of four turns around the receptacle. The six primordia of the calyx develop successively, passing around the receptacle in a clockwise manner. The primordia of the succeeding cycles develop in a like manner, except that they alternate with those of each preceding cycle.

3. Each zone later becomes completely meristematic, so that the floral organs of each whorl are laterally coalesced, so to speak. Finally the staminal and corolla zones become radially and laterally one continuous zone, so that the stamens appear to be adnate to, or outgrowths from, the corolla tube.

4. Each calyx lobe, corolla lobe, stamen, and carpel is supplied with one bundle from the vascular cylinder in the receptacle.

5. The calyx consists of a short tube surmounted by six linear to lanceolate lobes, 12–15 cell layers in thickness. The mesophyll is loosely arranged, and contains numerous chloroplasts.

6. The corolla is a short tube bearing six broadly lanceolate lobes, about eight cell layers in thickness.

7. The six stamens are connivent, and form a cone around the pistil. They may be said to be inserted on the throat of the corolla, but their primordia are at first free from it. Dehiscence is introrsely longitudinal.

8. The pistil is composed of six united carpels.

9. Glandular hairs are found on the calyx and corolla. Multicellular non-glandular hairs occur on the calyx, corolla, and style. All of the organs bear single celled papillary hairs. Stomata were found on the calyx, pedicel, and style, but not on the corolla or ovary.

The writer feels deeply indebted to Professor L. F. HEIMLICH for his generous assistance during the course of this investigation; to Professor E. J. KOHL who made the photomicrographs; and to Dr. M. W. GARDNER, through whose courtesy the material for study was obtained.

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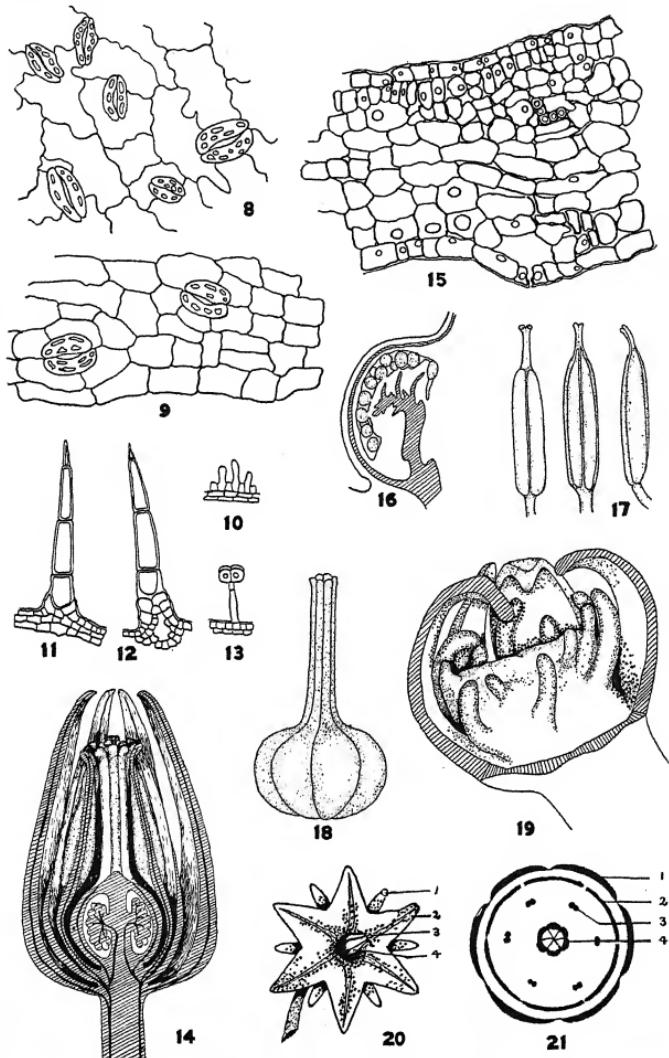
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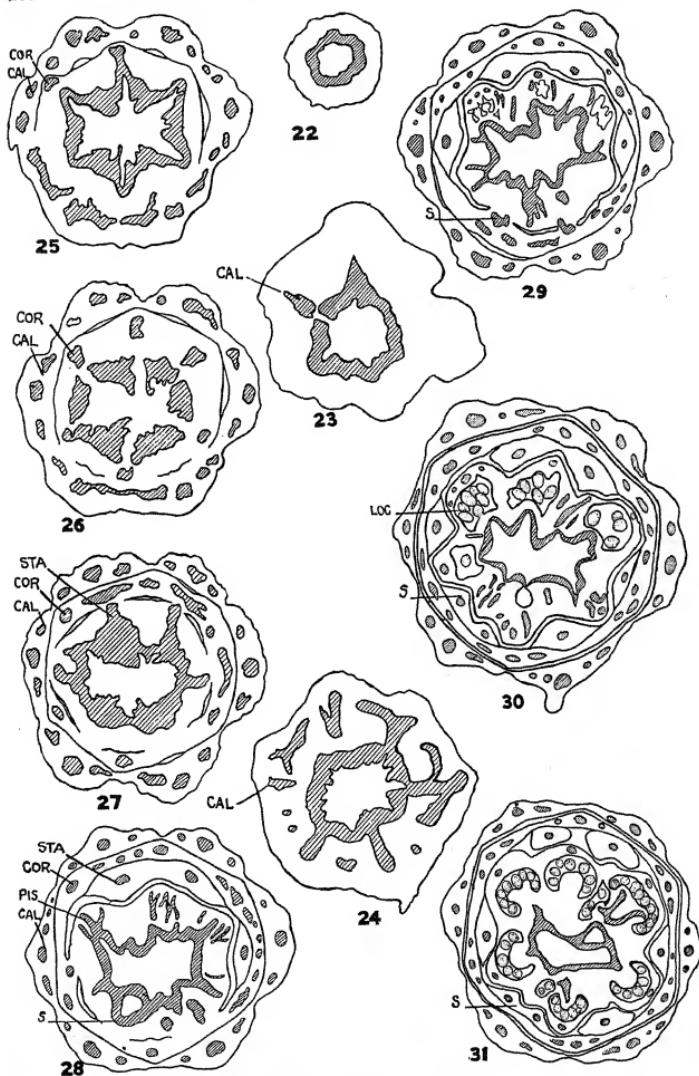
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EXPLANATION OF PLATES XI, XII

- FIG. 8.—Arrangement of stomata on dorsal surface of calyx lobe; $\times 250$.
- FIG. 9.—Arrangement of stomata on pedicel; $\times 250$.
- FIG. 10.—Papillae on surface of corolla; those on ovary similar; $\times 250$.
- FIGS. 11, 12.—Multicellular non-glandular hairs from dorsal surface of calyx; $\times 250$.
- FIG. 13.—Multicellular glandular hair from calyx; $\times 525$.
- FIG. 14.—Median longitudinal section through flower bud, drawn from fresh material; $\times 5$.
- FIG. 15.—Cross-section of calyx lobe, showing elevated stoma and substomatal cavity; $\times 250$.
- FIG. 16.—Longitudinal section through ovary, showing locule and forking vascular bundle (shaded); $\times 72$.
- FIG. 17.—Stamen in dorsal, ventral, and side views drawn from fresh material; $\times 3$.
- FIG. 18.—Pistil showing six united carpels; $\times 3$.
- FIG. 19.—Young flower bud with part of calyx removed to show developing primordia; reconstructed from series of drawings of longitudinal sections; $\times 125$.
- FIG. 20.—Tomato flower showing six-merous condition; drawn from fresh material.
- FIG. 21.—Diagrammatic plan of tomato flowers: 1, calyx lobe, 2, corolla lobe, 3, stamen, 4, carpel.
- FIG. 22.—Cross-section of pedicel just below receptacle, showing vascular cylinder (shaded); $\times 12$.
- FIGS. 23, 24.—Cross-sections through base of receptacle, showing origin of vascular bundles (*cal*) leading to calyx lobes; fig. 3 shows branching of each bundle; $\times 12$.



COOPER on TOMATO FLOWER



COOPER on TOMATO FLOWER



FIGS. 25, 26.—Cross-sections through receptacle just above traces leading to calyx, showing origin of traces (*cor*) leading to corolla; $\times 12$.

FIG. 27.—Vascular traces (*sta*) leading to staminal whorl; $\times 12$.

FIGS. 28, 29.—Vascular bundles (*pis*) leading to style and stigma; $\times 12$.

FIG. 30.—Locules (*loc*) formed in ovary, but vascular cylinder still intact; $\times 12$.

FIG. 31.—Vascular cylinder breaking up into branches leading to ovules; $\times 12$.

FIGS. 28, 29, 30, 31.—Series of sections showing at *S* development of two vascular bundles within one filament; $\times 12$.

SMOOTHNESS AND ROUGHNESS AND SPONTANEOUS
AGGLUTINATION OF BACTERIUM CITRI, BACT.
MEDICAGINIS VAR. PHASEOLICOLA, BACT. PHA-
SEOLI SOJENSE, AND BACT. TUMEFACIENS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 370

G. K. K. LINK AND KATHLEEN L. HULL

(WITH THREE FIGURES)

In the course of routine cultural work, and of observations incidental to agglutination tests of a series of experiments by LINK and LINK (6), and LINK and TALIAFERRO (7), data were obtained which appear significant in connection with the discovery by SHARP (10) of a smooth and rough strain of *Bact. phaseoli sojense*. SHARP reports isolation from a culture of *Bact. phaseoli sojense* of a strain whose colonies on agar media are smooth, and another whose colonies are rough. These, in harmony with the practice of animal bacteriologists, were designated as smooth (*S*) and rough (*R*) respectively. SHARP found further, in harmony with considerable data from the fields of medical and general bacteriology, that smoothness is directly correlated with motility, low agglutinability, and higher virulence, whereas roughness is directly correlated with low or no motility, higher agglutinability, and less virulence. He also found for the first time, in the rough strain, an instance of spontaneous agglutination of a schizomycete in distilled water. Finally, in harmony with data relative to some animal pathogenes, he found that high agglutinability was directly correlated in the acid range with low electricphoretic potential (P.D.), and low agglutinability with a high P.D. In preliminary experiments he, and later in more detailed work FALK, SHARP, and LINK (2), found for the first time that this correlation between agglutinability and P.D. does not exist in the alkaline range for either the smooth or the rough strain.

This paper is a report of data which have a bearing upon this interesting and apparently important discovery by SHARP. A discussion of the full significance of these phenomena in the light of data

from the fields of general and medical bacteriology is reserved for a later and more detailed paper.

The original sources of the cultures used are: *Bact. citri* (E. F. SMITH, 1927), *Bact. medicaginis* var. *phaseolicola* (W. H. BURKHOLDER), *Bact. phaseoli* *sojense* (R) (SHARP, 1926), and *Bact. tumefaciens* (A. J. RIKER, 1925). The immediate source of the cultures of *Bact. tumefaciens* were isolations from galls produced upon inoculation of tomatoes by Miss HULL with a culture which SHARP had isolated from tomato galls following inoculation with the original culture.

In the course of routine classwork with *Bact. tumefaciens*, Miss HULL found it impossible to follow SMITH's (12) advice to select only colonies which come up smooth and glistening and remain translucent. She obtained colonies which were opaque and wrinkled at first, and only later became smooth and glistening. Such colonies upon inoculation of tomato plants consistently developed typical galls. She and other students also had found that cultures from such colonies gave the cultural tests characteristic of *Bact. tumefaciens*. This experience was forgotten until agglutination tests were made by LINK and LINK (6) and LINK and TALIAFERRO (7), in which *Bact. tumefaciens* antiserum and suspensions of this organism were used. The first tests were run with the normal serum of animals selected for immunization. An even suspension of *Bact. tumefaciens* had been obtained following three washings in 0.85 per cent NaCl solution and centrifugation for 45 minutes at 3000 revolutions per minute. When the agglutination tests were read, it was found that while there had been flocculation in all tests at all dilutions in which *Bact. tumefaciens* was the test antigen, most of the suspended organisms still were in suspension. The controls in saline solution had behaved identically. Fortunately some of the excess stock suspension of *Bact. tumefaciens* had been kept in the ice chest over night, and it was found that in this, too, there had been beautiful flocculation of a fraction of the organisms (about one-fourth), and that the remainder were still in suspension. In reflecting upon the situation and casting about for a possible explanation, the experience of Miss HULL was recalled in the light of SHARP's findings.

It was thought possible that we were again dealing with an organism which showed the phenomenon of smooth and rough colo-

nies. More detailed work growing out of this working hypothesis has revealed that on potato-dextrose or beef-dextrose agar a culture of the strain of *Bact. tumefaciens* of tested pathogenicity comes up in colonies, about 50 per cent of which are either entirely wrinkled and opaque or only partly so, while the other half consists of smooth translucent colonies (figs. 1, 2). However, the rough colonies develop smooth shiny translucent margins, and after three days on potato-dextrose agar, and after five days on beef-dextrose agar, the colonies appear smooth and shiny and translucent as described by SMITH, although the opaque centers remain visible (fig. 3). On agar

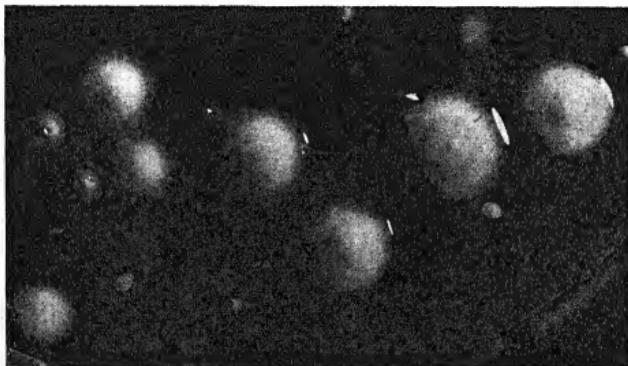


FIG. 1.—Typical smooth surface colonies of *Bact. tumefaciens* on beef-dextrose poured plates, six days old; $\times 2$.

slants the *S-R* cultures remain rough at the margins where new growth is most intense for four or five days before becoming entirely smooth. The rough colonies are very compact and firm, so that when transfer of a portion of them is attempted by needle insertion, the entire colony usually is lifted from the agar surface. Upon similar procedure with a smooth colony only a slight amount of the colony adheres to the needle.

In the course of the tests it was noted that colonies of *Bact. citri* and *Bact. medicaginis* var. *phaseolicola* developed what appeared to be roughness. In shake dilutions of *Bact. citri* in beef-dextrose agar or in potato-dextrose agar colonies appear which are smooth and rough, but more rough than smooth, and which become smoothulti-

mately. On potato agar the colonies are more rough than on beef agar. These colonies, however, are not as strikingly rough as those of *Bact. phaseoli sojense* or *Bact. tumefaciens*, and appear more like those described by SMITH as having internal convolutions.

Bact. medicaginis var. *phaseolicola* (BURKHOLDER'S no. 35) always gave smooth colonies. Strain no. 23, however, which BURKHOLDER considered identical with no. 35, develops smooth-rough colonies on potato-dextrose and beef-dextrose agar. BURKHOLDER (1) describes this organism as having colonies on nutrient agar that are concentrically ringed with "edges undulate." On gelatin the

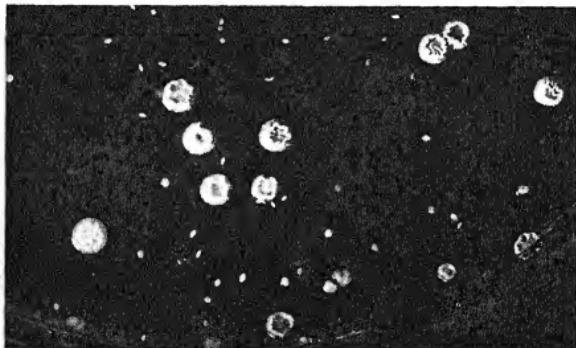


FIG. 2.—Typical rough colonies of *Bact. tumefaciens*, four days old; $\times 2$

colonies are raised and "somewhat wrinkled." The colonies are butyrous at first but later become brittle. *Bact. phaseoli sojense* (*R*), in contrast with *Bact. citri* and *Bact. tumefaciens*, comes up smooth and becomes and remains rough on both potato-dextrose and beef-dextrose agar.

These observations would seem to indicate that smoothness and roughness are not absolutely qualitative differences, but that they have a quantitative element in them. Apparently there are intergrades, of which perfect smoothness is one extreme and extreme roughness the other. This is borne out by data relative to the agglutinability of the organisms under discussion.

SHARP found that the *R* strain of *Bact. phaseoli sojense*, which consists of extremely rough but butyrous colonies, agglutinated

spontaneously in both distilled water and in 0.85 per cent NaCl solution, and that nine washings in distilled water did not render it suspensible. LINK and LINK (6) three months later, dealing with subcultures from the same culture, found that the organism still agglutinated spontaneously in 0.85 per cent NaCl solution, but that after resuspension and washing, a permanent suspension could be obtained which was used by them in agglutination tests. This may indicate that the organism is in an unstable condition at present, and that reversion may be taking place.

Bact. citri and *Bact. medicaginis* var. *phaseolicola* no. 23, which develop partially rough colonies, when suspended in 0.85 per cent

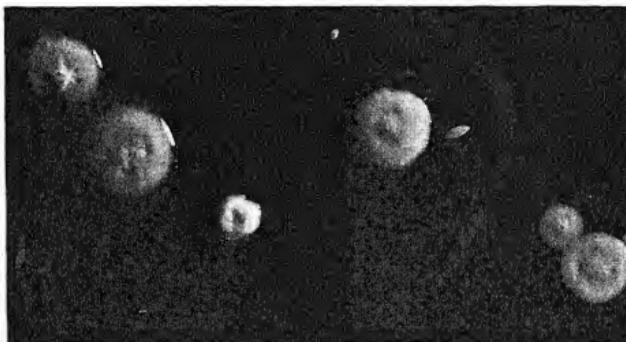


FIG. 3.—Transition colonies of *Bact. tumefaciens*, showing rough center and smooth margin, six days old; $\times 2$.

NaCl solution do not settle out, even after 36 hours. On the other hand, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. flaccumfaciens*, *Bact. phaseoli sojense* (S), and *Bacillus carotovorus* (*Iris*), which show no evidence of roughness in the cultures, settled out slightly from suspensions after 24 hours, while suspensions of the smooth organisms *Bact. campestris*, *Bact. tumefaciens*, *B. carotovorus* (3a), and *B. aroideae* settled out heavily. These data indicate that apparently there is not always a definite direct correlation between roughness and ready flocculation, and smoothness and less ready agglutination. Further work may show, however, that there are various types of roughness and smoothness, and that agglutinability is correlated with a definite type of roughness.

Bact. tumefaciens seems to be the most unstable organism so far studied. Interest in this apparent instability is enhanced by the discovery, by LINK and LINK (6) and LINK and TALIAFERRO (7), of what may prove to be a case of serological cosmopolitanism when antisera of various organisms are tested against suspensions of *Bact. tumefaciens*. *Bact. tumefaciens*, like *Bact. phaseoli sojense* (*R*), agglutinates spontaneously in distilled water. Suspensions in distilled water of colonies which appear entirely (or mostly) rough give almost complete clearing of the supernatant liquid in 12 hours, while suspensions made from older colonies which appeared smooth had many organisms in suspension after 12 hours, although there also was a heavy precipitate.

Whether such correlation between character of colony agglutinability and virulence exists for these organisms, as has been described by SHARP for *Bact. phaseoli sojense*, remains to be determined. Investigations are under way on this problem. Because of the possibility that in *Bact. tumefaciens* we are dealing with an organism in which an intermediate form is changing into the *R* and *S* forms, or the *R* form is giving rise to an intermediate or smooth form, the results of these studies are awaited with much interest.

SMITH (12) has used pathogenicity and production of specific symptoms as important criteria for species identification. Serological work (5, 6, 7, 10) with pathogenes which are not readily differentiated culturally, but which show host specificity, indicate strongly that SMITH was correct in his theory. In the light of these facts and the data here reported for *Bact. tumefaciens*, the directions which SMITH has given for isolation of this organism are very significant. He states:

.... and all circular white colonies, if opaque, are negligible. Only those colonies that come up slowly, that remain for a considerable time small, circular, raised and glistening-translucent (watery) need be considered, and. Some colonies selected for the subcultures may not prove to be infectious it is advised to experiment with quite a number of colonies, rejecting all that show a narrow clear zone about the colonies.

He also refers to the observation that colonies with a thin, wrinkled, and dull surface develop on agar after passage of the organism through peptone-bouillon.

There are other references in the literature which indicate that *Bact. tumefaciens* is unstable, both as to form and virulence. SMITH, BROWN, and TOWNSEND (11), RIKER (8), and ROBINSON and WALKDEN (9) ascribe various sizes to *Bact. tumefaciens*. SMITH has repeatedly referred to involution forms:

It passes over easily (under action of cold, sodium chloride, or acids) into club-shaped, Y-shaped, and variously branched involution forms, which often are dead or dying, i.e., will not grow on agar-poured plates, or come up slowly. These moribund involution forms occur not only in culture media, but are common in the tumor, and to them must be attributed not only our former difficulty in isolating the organism, but also the failure of others to isolate.

LEVINE (4) goes a great deal further, and reports decided changes in form of *Bact. tumefaciens* which suggest the phenomenon of cyclogeny. He refers to the appearance of jelly-like substances of amorphous form which appear in old cultures, and which upon transfer to new media give rise to new growth in which long rods develop, some of them of beaded appearance. The rods are reported to break up, until finally small faintly staining cocci with bacilli or filaments appear. The cocci finally give way to the amorphous masses first described.

One is moved to query how many other similar phenomena might be discovered if all plant pathogens were studied as critically as *Bact. tumefaciens* has been. There are some references to colony instability (not so termed in the literature) which indicate that the phenomenon probably is as frequent among plant pathogens as it is among the forms studied by the general and medical bacteriologist. SMITH's description and illustrations of the "windowed" colonies of *Bact. malvacearum* are significant. They may represent erosion phenomena. The colonies pictured in fig. 250, to show the effect of freezing, look as rough as the rough colonies of *Bact. phaseoli sojense*. The remarkable series of photographs of colonies of *Bact. translucens* var. *undulosum* showing liquefaction pits are very suggestive of lytic phenomena. GARDNER and KENDRICK (3) describe aberrant colonies of *Bact. viridis faciens*, and illustrate one with lobed margin and surface sculpturing.

Conclusions

1. The phenomenon of roughness and smoothness of colony form is reported for *Bact. citri*, *Bact. medicaginis* var. *phaseolicola* no. 23, and *Bact. tumefaciens*.

2. These organisms seem to be in a state of instability so far as these characters are concerned, *Bact. tumefaciens* seeming to be the least stable.

3. In *Bact. tumefaciens* roughness in colony form seems to be correlated directly with ready spontaneous agglutination in distilled water, and in 0.85 per cent NaCl solution, whereas smoothness seems to be directly correlated with less agglutinability. Mixed cultures can be separated roughly by means of this differential agglutination.

4. These phenomena of colony form and agglutination seem to be qualitative and quantitative or perhaps merely quantitative.

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CYTOLOGICAL STUDY OF STIGONEMA MAMMILOSUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 371

SYBEL LEE

(WITH PLATE XIII)

The Cyanophyceae, whether considered as the lowest of the algae, or as closely related to the bacteria, are interesting on account of primitive features which might give investigators a better understanding of the cells in higher plants; while the central body might help to solve the problem of the origin of the nucleus.

In 1904-1905 OLIVE (5), who studied *Oscillatoria* and other species of Cyanophyceae, came to the conclusion that the central body is a nucleus with mitotic division not essentially different from that in higher plants. GARDNER (1) reported that the Cyanophyceae contain a nucleus which occupies a relatively large portion of the cell, but which divides amitotically, except in *Synechocystis*, which possesses a primitive form of mitosis. HAUPT (2), studying *Anabaena*, came to the rather surprising conclusion that the cell of the Cyanophyceae has no real nucleus, although the central substance resembles chromatin and may have similar functions.

Although the Cyanophyceae have been investigated exhaustively by many cytologists there is no general agreement. Since *Stigonema* was available and had received little more attention than a taxonomic treatment, Professor CHARLES J. CHAMBERLAIN, to whom I am indebted for advice and criticism, suggested that I examine this genus.

Material and methods

Material was collected in 1920 at Mount Desert, off the coast of Maine. It was fixed in a weak Flemming solution and was brought up to the 70 per cent alcohol by Dr. WM. R. TAYLOR, who identified the species as *Stigonema mammillosa*. Since *Stigonema* is a simple plant, material mounted whole will show the general features; but sections cut from 3-8 μ are better for details.

The Venetian turpentine method was used for the whole mounts. Iron-alum haematoxylin is good for cell contents, but crystal violet is a better stain for the sheath. For sections, safranin followed by Orange G in clove oil was tried, but was not as good as iron-alum haematoxylin for the differentiation of cell contents. Crystal violet was also used in sections for differentiating the sheath.

Topography

Taxonomically *Stigonema* is classified under the filamentous forms with true branching, but the cells are aggregated more or less like colonies. In the same filament each group has cells in different stages, so that their appearance in the living condition and their reactions toward the stains are different, some groups of cells staining deeply while the others stain very lightly, producing a spotted appearance.

There is true branching, but it is irregular (figs. 1, 2). The smallest branches are only one cell in thickness (fig. 3), while the largest reach ten cells in diameter. The cells of the young branches always stain alike, as shown in figs. 3 and 4; consequently these cells are more or less in the same stage. Some of the cells in the older filaments become rejuvenated while the rest remain old, so that there are different conditions in the same filament. The outer cells are most likely to rejuvenate and give rise to new branches, while the rejuvenation of inner cells gives rise to new groups. In the limited space and the struggle for existence the weakest cells and groups of cells degenerate (figs. 3, 6). The vigorous groups of cells, abundantly supplied with chlorophyll and phycocyan, contrast sharply with the weaker and degenerating cells, and thus cause the spotted appearance which gives the genus its name.

A thick gelatinous mass, the sheath, envelops the cells, except in very young filaments where the cells are in close contact with one another, so that the whole plant is much like a loose association of colonies. The sheath about single cells and groups of cells is often distinctly lamellated.

There are four kinds of cells: the heterocyst (fig. 2), concave cells (fig. 2), degenerating cells (figs. 3, 6), and the active vegetative cells.

Vegetative cells

The vegetative cells of *Stigonema* are spherical in shape, because they are free from pressure of neighboring cells, on account of the intrusion of gelatinous secretions. The cells are almost as free from one another as if the plant were unicellular; however, the cells of each group are connected by protoplasmic connections, which are often quite conspicuous (fig. 7).

There are no chromatophores, the protoplasm occupying the entire space between the central body and the cell wall. It is differentiated into two regions, a peripheral and an inner region, the former darker than the latter, because of the coloring matters (figs. 5, 6, 8). The protoplasm of the resting cells is more or less vacuolated, but that of the active cells seems nearly homogeneous.

The so-called central body is a nucleus. It occupies a relatively large portion of the cell, and is sharply marked off from the protoplasm in spite of the absence of a nuclear membrane (fig. 8). It is composed of an achromatic portion and chromatic granules. The chromatic portion is composed of an unusually dense substance, consisting of a more or less definite number of chromatic granules (figs. 8, 9), which become aggregated into rodlike bodies resembling chromosomes when the nucleus divides (figs. 10-15). Two groups of these bodies are formed, but they do not split lengthwise and divide equally like chromosomes in the higher plants.

While the central body is a naked nucleus, it should be regarded as the homologue of the true nucleus of the higher plants. It is more primitive and does not show such a precise division and distribution of the chromatic material, but there is an aggregation of chromatic granules to form a spireme stage, which is followed by a segmented stage (figs. 10-12). The spireme and the segmentation resemble somewhat the behavior of the nucleus as described by KOHL (4) for *Tolypothrix*; but the spireme is not so complete nor distinct as shown in his figures. There is also some resemblance to the figures in HAUPt's paper on *Anabaena*, where we should interpret the deeply staining strands in the central body as chromatin. The arrangement of these segments into two groups follows, and cell division takes place (figs. 13-16).

Nothing was found which might be identified as a spindle or spindle fibers, nor was any formation of a cell wall discernible in the region where a spindle might be anticipated; but after nuclear division the elongation of the cell takes place, and is followed by the appearance of a cleavage furrow which becomes deeper and deeper until the cell itself is separated into two. Nuclear division always precedes the appearance of cleavage, because when the chromatic groups separate they leave a less resistant region in the outer wall, which gradually presses in until the daughter cells are formed. After that the nuclei of the daughter cells break up into chromatin granules (figs. 17, 18).

The cells of the resting stage contain a great number of accumulated food granules, which will be used when the cells are rejuvenated. The chromatic substance of the central body becomes condensed into a single deeply stained body suspended by its radiations in the center of the cell (figs. 19, 20). No granules could be distinguished in this dense body, which looks as homogeneous as the nucleus of *Porphyra*, as figured and described by ISHIKAWA (3).

Conclusions

1. The cells of *Stigonema* act independently, although aggregated into a filament which is often many cells in diameter.
2. When a cell is rejuvenated at the surface of the filament it produces a true branch; when the rejuvenating cell is more deeply placed it produces a group of vigorous cells, which, being surrounded by comparatively inactive cells, gives the filament a spotted appearance, which suggested the generic name.
3. The central body is a primitive nucleus which has no nuclear membrane or nucleolus, and no spindle during division.

[Accepted for publication January 27, 1927]

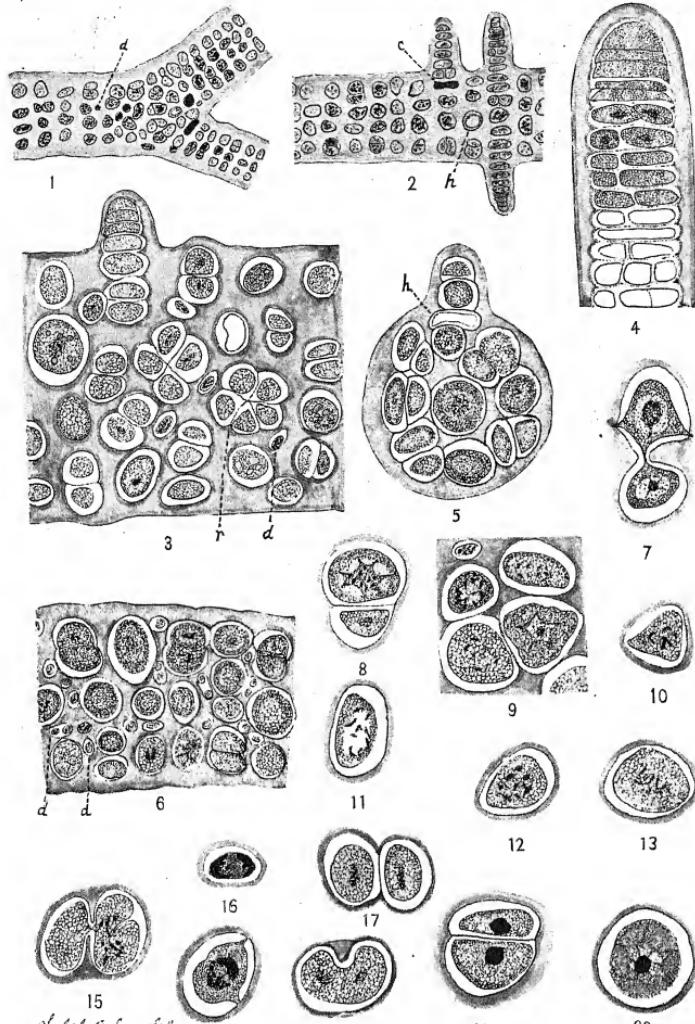
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DESCRIPTION OF PLATE XIII

- FIG. 1.—Portion of filament mounted whole; $\times 65$.
- FIG. 2.—Old branch with three young branches: *c*, concave cell; *h*, heterocyst; $\times 65$.
- FIG. 3.—Adult branch with young branch and group of cells from a rejuvenating cell (*r*); $\times 532$.
- FIG. 4.—Young branch somewhat older than those in figs. 2 and 3; $\times 532$.
- FIG. 5.—Transverse section of adult branch with young one; $\times 532$.
- FIG. 6.—Adult branch with numerous degenerating cells; $\times 532$.
- FIG. 7.—Two cells, lower showing central body with radiations and upper showing connection between cells; $\times 1080$.
- FIG. 8.—Two cells, upper showing central body with radiations and chromatin granules; $\times 1080$.
- FIG. 9.—Several cells showing aggregation of granules; $\times 1080$.
- FIG. 10.—Aggregation of chromatin granules into rodlike chromat in masses; $\times 1080$.
- Figs. 11, 12.—Cells with nucleus showing rodlike chromat in masses; $\times 1080$.
- FIG. 13.—Cell with chromatin beginning to divide into two groups; $\times 1080$.
- FIG. 14.—More advanced stage in division; $\times 1080$.
- FIG. 15.—Chromatin in two groups and furrows dividing the cells; $\times 1080$.
- FIG. 16.—Advanced stage in nuclear division; $\times 1080$.
- FIG. 17.—Division completed; $\times 1080$.
- FIG. 18.—Two groups of chromatin and beginning of cleavage furrow; $\times 1080$.
- FIG. 19.—Division completed and chromatin in homogeneous mass; $\times 1080$.
- FIG. 20.—Nucleus with homogeneous chromatin and radiating strands; $\times 1080$.



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BRIEFER ARTICLES

ISOSTIGMA PEUCEDANIFOLIUM (SPRENG.) LESS., A VALID NAME

In a former article (BOT. GAZ. 81:241-257. 1926) I presented a revision of the entire genus *Isostigma* Less. At that time an extended discussion of *I. peucedanifolium* (Spreng.) Less. was given (*i.e.* pp. 252-254). A specimen borrowed from the Delessert Herbarium, and which had seemed to be authentic for *Bidens megapotamica*, was discussed in detail. It was stated that this specimen was of the species widely known as *Isostigma peucedanifolium*. As my repeated searches in Paris for the real type of *B. megapotamica* Spreng. all had ended in failure, it seemed probable that no type had been preserved among SPRENGER's specimens, and that therefore the apparent cotype in the Delessert Herbarium would have to suffice. Under the compulsion incident to giving a monographic treatment of the genus the trivial name *megapotamica* was adopted as the earliest for *Isostigma peucedanifolium* (Spreng.) Less., and the resulting new combination *Isostigma megapotamicum* was employed.

In the spring of 1926, I was submitted for advance perusal and criticism some preliminary pages of manuscript upon the genus *Thelesperma*, by S. F. BLAKE, Associate Botanist of the United States Bureau of Plant Industry. It was a pleasant surprise to find at one point in the reading of his treatment that BLAKE had actually found the type itself for *Bidens megapotamica* Spreng. While searching at Paris (Herb. Mus. Hist. Nat.) for various Compositae in other genera, he had found this type, mounted upon the same sheet with the type of *Tagetes fusclosa* Spreng. Of these two specimens he obtained a small but excellent photograph. This illustration confirms BLAKE's statement (*in litt.*) that the type of *B. megapotamica* Spreng. "is a plant which is clearly *Thelesperma scabiosoides*." This being the case, the name *megapotamica* belongs in *Thelesperma*, where the combination *Thelesperma megapotamicum* (Spreng.) O. Ktze. must stand, and the name *T. scabiosoides* Less. must reduce to a synonym, as stated once before (BOT. GAZ. 76:91. 1923). *Isostigma peucedanifolium* (Spreng.) Less. must remain, therefore, the valid name for the species numbered 9 in my Revision (*i.e.* 252). —EARL EDWARD SHERFF, Chicago, Ill.

CURRENT LITERATURE

BOOK REVIEWS

Photosynthesis

A very valuable summary of the facts now known about photosynthesis, and the theories proposed to account for it, has been prepared by SPOEHR.¹ There are seven chapters in the book, with general headings as follows: Origin of organic matter and the cosmical functions of green plants; Nature of photosynthesis as determined by observations of gaseous interchange and the origin of organic matter; Products of photosynthesis; Methods of measuring photosynthetic activity; Chemistry of photosynthesis; Energy relations in photosynthesis; and Chlorophyll and chloroplasts.

The first chapter particularly is of general interest, and should be read by every teacher of botany and by every student who wants to know something of the significance of botanical science. The succeeding chapters are somewhat more technical, of course, but the book is quite readable to those who are familiar with organic chemistry; and it will no doubt stimulate a great deal of interest in the unsolved problems connected with the synthetic activities of plants. To students and workers especially interested in the physiology of plants, the chapters on the products, methods of measuring, and the chemistry of the process will be most attractive.

The chapter on energy relations is based largely upon the work of BROWN and his coworkers ESCOMBE and WILSON. Unfortunately for the work of these pioneers in the study of energy relations, excellent as it seems to be on first examination, they considered the reflection of light from leaves negligible, and worked out their ideas of the quantitative utilization of sunlight energy by the leaves of plants from this point of view. The reviewer has been engaged recently in measuring the reflection of energy in the form of light from the surfaces of leaves, and finds that a much larger proportion of the energy than is usually assumed may be reflected from the surfaces of leaves. In the darker portions of the spectrum, the shortest violet and longest red rays, the reflection may run as low as 3 per cent, but in the brightest green region of the spectrum, depending upon the individual leaf color, the reflection of light at an angle normal to the surface is seldom less than 8 per cent, and may run as high as 20 per cent in light green vegetation such as is seen in early spring. Autumn colored leaves and albino leaves reflect still larger percentages of the incident energy, even exceeding 50 per cent in some cases. These results throw grave doubts upon the accuracy of the BROWN and ESCOMBE figures, in which they worked out a 100 per

¹ SPOEHR, H. A., Photosynthesis. 8 vo. pp. 393. Chemical Catalog Co. New York. 1926.

cent balance sheet of the energy income and outgo of leaves, on the supposition that reflection is negligible. This chapter, then, deals with a phase of the subject which needs reinvestigation, and a rewriting *in toto*. The fundamental facts are needed before we can draw a reasonable picture of the energy relations of the plant.

The book is so well written, and the materials so admirably marshalled that one dislikes to point out errors of judgment. However, there is one feature in chapter II which calls for comment. In connection with gaseous exchange the author considers the photosynthetic quotient, and incidentally the respiratory quotient of plants. He points out the confusion in the literature over the matter of designating these ratios, and then adds to the confusion by choosing the wrong designation. The animal physiologists have for a long time used $\frac{CO_2}{O_2}$ to designate the respiratory ratio, and this has been at least common among plant physiologists. There is no indication that the animal physiologists will ever change their method, and they consider it rather absurd to write the ratio $\frac{O_2}{CO_2}$. There are many reasons why plant and animal physiologists should use common symbols for the processes that are common to plants and animals, as respiration is. The reviewer considers it an unfortunate error in judgment to use $\frac{CO_2}{O_2}$ as the photosynthetic quotient, and $\frac{O_2}{CO_2}$ as the respiratory quotient. Certainly this method will not be followed by animal physiologists, and it ought not to be by plant physiologists.

A few typographical errors occur, as one might expect to find even in the best of books. The most striking instance noted by the reviewer in reading the book was on page 131, where the word calorie occurs as "callory" and "calory" in consecutive lines. If the book should reach a second printing a number of errors, including these, should be eliminated.

Taking the book as a whole, it is one of the best American monographs so far published in the field of plant physiology. It is hoped that the reception given it will encourage the preparation of other valuable monographs in this field.—C. A. SHULL.

Surface chemistry

The rapid development of the field of surface phenomena is emphasized by the appearance of a book by RIDDELL² on surface chemistry. The reactions, the equilibrium relations, and the chemical structure of interfaces must be thoroughly understood before we can grasp the essential features of adsorption, enzyme and other catalysts, and the causes of stability in colloidal gel and sol systems. Beyond this are the phenomena of life, anabolic and catabolic changes, the

² RIDDELL, E. K., An introduction to surface chemistry. 8vo. pp. x+336. Cambridge University Press. Cambridge, England.

effects of anaesthetics and other surface-active chemicals on the living colloidal systems of organisms. These phenomena can only be understood properly in the light of interphase chemistry and physics.

The book by RIDEAL draws together the facts concerning the molecular structure and kinetics of the two dimensional world represented by phase surfaces, as developed by the investigations of such pioneers as LORD RAYLEIGH, MARCELIN, HARDY, LANGMUIR, and others. The author, as a follower in the footsteps of these earlier students of surface phenomena, has already contributed in excellent fashion to the advancement of this field. He sets forth the discussion in nine chapters, which take up the surface tension of liquids, surface tension of solutions, surface films of insoluble materials, liquid-liquid interfaces, the gas-solid interface, liquid-solid interface, difference of potential at interfaces, conditions of stability in suspensions and emulsions, gels and hydrated colloids.

The first seven chapters lay down an admirable basis for the understanding of the last two chapters, which will be most interesting to students of biology. In the chapter on stability of suspensions and emulsions there is a good discussion of Brownian movement, electrolytic coagulation, adsorption of ions, and rules of precipitation of colloids. The effects of non-electrolytes on suspensions, and the precipitation of metallic sols by minute quantities of protective colloids are given brief treatment at the close of this chapter.

The final chapter on gels and hydrated colloids considers the theories of gel structure, structure of gelatin, rigid and moist gels, and the properties of such gels as silica gel, gelatin, soaps, and colloidal dyes. As DONNAN, who writes the preface, remarks, "every student and investigator of surface and colloid phenomena owes Dr. RIDEAL a warm debt of gratitude for his admirable survey and presentation of a great and rapidly advancing field of physico-chemical science."

—C. A. SHULL.

Economic botany

One of the most attractively written books on botany that the reviewer has ever read is one by PEATTIE¹ on the important crop plants of the world. The volume might well be styled romantic tales of economic plants. Starting with the rather well known history of the spice trade, the author tells in lucid language of the important part played by many crops in the history of man. Suggestive chapter titles are: quinine, the coming of a savior; the age of rubber; camphor, the strategic crop; the potato, the poor man's friend; the poppy, blessing and curse; tobacco, the companionable weed. It is shown quite conclusively that plants quite as much as other things have determined the rise and fall of nations. In the final chapter, entitled "Must we starve?", it is shown that the key to the future of mankind resides in plants. To one who does not believe in the significance of botany, this book must indeed be a potent revealer of new truth. The figures are quaint maps depicting modern facts in the garb of the middle ages.—H. C. COWLES.

¹ PEATTIE, D. C., *Cargoes and harvests*. 8vo. pp. 311. figs. 16. New York: D. Appleton and Co. 1926.

NOTES FOR STUDENTS

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⁵ SPRAGUE, T. A., and SANDWITH, N. Y., New species of *Strychnos* from Tropical America. Kew Bull. Miscell. Inform. no. 3. 127-133. 1927.

⁶ BUSH, B. F., The glabrate species of *Tilia*. Bull. Torr. Bot. Club 54:231-248. 1927.

⁷ GLÜCK, H., A new *Sagittaria* from Florida. Bull. Torr. Bot. Club 54:257-261. 1927.

⁸ MERRILL, E. D., New Chinese ligneous plants. Jour. Arnold Arboretum 8:3-19. 1927.

⁹ CROW, W. P., *Crinalium*, a new genus of Cyanophyceae, and its bearing on the morphology of the group. Ann. Botany 4x:161-165. 1927.

¹⁰ FERNALD, M. L., Two summers of botanizing in Newfoundland. Contrib. Gray Herb. 76. pp. 144. 1927.

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effects of anaesthetics and other surface-active chemicals on the living colloidal systems of organisms. These phenomena can only be understood properly in the light of interphase chemistry and physics.

The book by RIDEAL draws together the facts concerning the molecular structure and kinetics of the two dimensional world represented by phase surfaces, as developed by the investigations of such pioneers as Lord RAYLEIGH, MARCELIN, HARDY, LANGMUIR, and others. The author, as a follower in the footsteps of these earlier students of surface phenomena, has already contributed in excellent fashion to the advancement of this field. He sets forth the discussion in nine chapters, which take up the surface tension of liquids, surface tension of solutions, surface films of insoluble materials, liquid-liquid interfaces, the gas-solid interface, liquid-solid interface, difference of potential at interfaces, conditions of stability in suspensions and emulsions, gels and hydrated colloids.

The first seven chapters lay down an admirable basis for the understanding of the last two chapters, which will be most interesting to students of biology. In the chapter on stability of suspensions and emulsions there is a good discussion of Brownian movement, electrolytic coagulation, adsorption of ions, and rules of precipitation of colloids. The effects of non-electrolytes on suspensions, and the precipitation of metallic sols by minute quantities of protective colloids are given brief treatment at the close of this chapter.

The final chapter on gels and hydrated colloids considers the theories of gel structure, structure of gelatin, rigid and moist gels, and the properties of such gels as silica gel, gelatin, soaps, and colloidal dyes. As DONNAN, who writes the preface, remarks, "every student and investigator of surface and colloid phenomena owes Dr. RIDEAL a warm debt of gratitude for his admirable survey and presentation of a great and rapidly advancing field of physico-chemical science."

—C. A. SHULL.

Economic botany

One of the most attractively written books on botany that the reviewer has ever read is one by PEATTIE³ on the important crop plants of the world. The volume might well be styled romantic tales of economic plants. Starting with the rather well known history of the spice trade, the author tells in lucid language of the important part played by many crops in the history of man. Suggestive chapter titles are: quinine, the coming of a savior; the age of rubber; camphor, the strategic crop; the potato, the poor man's friend; the poppy, blessing and curse; tobacco, the companionable weed. It is shown quite conclusively that plants quite as much as other things have determined the rise and fall of nations. In the final chapter, entitled "Must we starve?", it is shown that the key to the future of mankind resides in plants. To one who does not believe in the significance of botany, this book must indeed be a potent revealer of new truth. The figures are quaint maps depicting modern facts in the garb of the middle ages.—H. C. COWLES.

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¹⁰ FERNALD, M. L., Two summers of botanizing in Newfoundland. Contrib. Gray Herb. 76. pp. 144. 1927.

land or of Eastern America were discovered. The collections are listed, together with some revisions. There are given also full citations of collections elsewhere, thus indicating the geographical distribution. There are 13 new species described, 3 of which belong to *Salix*, and also 9 new varieties.

JOHNSTON,¹¹ in continuation of his studies of the Boraginaceae, has published a revision of the American Boraginoideae known from south of Panama. There are 17 genera presented, one of which (*Nesocaryum*) is described as new, and 86 species. Much the largest genera are *Cryptantha*, with 40 species, and *Plagio-bolrys* with 22. There are 11 new species described and many new combinations.

RYDBERG,¹² in continuation of his studies of the Fabaceae, has published an account of *Hamosa*, a genus of 20 species, 13 of which he has segregated from *Astragalus*, and 4 of which are described as new.

RIDLEY,¹³ in concluding his presentation of *Argostemma*, describes 15 new species and 4 new varieties, and also establishes a new genus, *Argostemmella*, with 2 species from Borneo.—J. M. C.

Biological Abstracts.—The first number of this publication has appeared, bearing the date December 1926. It contains abstracts of 1878 titles, representing the whole field of the "World's literature in theoretical and applied biology, exclusive of clinical medicine." Of the 1878 titles, 63 are general, 109 are in bacteriology, 700 are in botany, and 1006 are in zoology. The subjects covered in the more general field are biography, history, and bibliography, general biology, evolution, cytology, genetics, biometry, and ecology. The subjects in the field of plant science are phytopathology, plant physiology, biochemistry and biophysics, systematic botany, morphology and anatomy of vascular plants, paleobotany, pharmacognosy and pharmaceutical botany, forestry, horticulture, and agronomy. The organization of the material is quite complete, so that papers and authors are very accessible. As stated in the preface, the necessity for such a guide to current literature is due to "the increasing interdependence of the various fields of biological science, coupled with the constantly growing volume and complexity of the literature." This publication should certainly be accessible to every botanist. It will appear monthly, the volume closing the calendar year with a concluding index. This is a great cooperative enterprise, and biologists throughout the world are expected to contribute abstracts. Although the subscription price is \$15.00 a year, a special price of \$9.00 is made for all personal subscriptions. The editorial and executive office is located at the University of Pennsylvania, Philadelphia.—J. M. C.

¹¹ JOHNSTON, I. M., A revision of the South American Boraginoideae. Contrib. Gray Herb. 78. pp. 118. 1927.

¹² RYDBERG, P. A., Notes on Fabaceae. VIII. *Hamosa*. Bull. Torr. Bot. Club 54: 13-23. 1927.

¹³ RIDLEY, H. N., The genus *Argostemma*. Jour. Bot. 65:33-41. 1927.

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